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Selected Abstracts
on

ONCOGENE PROTEIN PRODUCTS

R.A. Weinberg, Ph.D.
Consulting Reviewer

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INTRODUCTION

This ONCOLOGY OVERVIEW on proteins of retroviral and cellular oncogenes includes abstracts selected from the CANCERLIT database referencing articles published mainly from 1982 to mid-1986. The last CANCERLIT update searched for this ONCOLOGY OVERVIEW was June, 1986.

THE STRATEGY USED TO RETRIEVE THESE ABSTRACTS CAN BE REQUESTED FROM THE CIDAC-CCB AND ENTERED ON ANY MEDLARS/MEDLINE TERMINAL SYSTEM TO UPDATE REFERENCES IN THIS AREA.

The scope of this ONCOLOGY OVERVIEW covers the protein products of cellular and retroviral oncogenes. The protein products are organized by their localization within the cell, whether cytoplasmic or nuclear. The protein products are further organized within the OVERVIEW by their activity and their homology with other oncogene protein products. Reviews and other related studies are also included.

This ONCOLOGY OVERVIEW was prepared by Phyllis Guinivan, Ph.D., Subject Specialist for the CIDAC for Carcinogenesis and Cancer Biology, Information Ventures, Inc., Philadelphia, PA. Robert A. Weinberg, Ph.D. of Whitehead Institute for Biomedical Research, Cambridge, MA, was the Consulting Reviewer and wrote the Editorial Commentary.

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EDITORIAL COMMENTARY

Work on cellular oncogenes has changed dramatically in the ten years since their discovery. It was in 1976 that workers in the laboratory of Harold Varmus and Michael Bishop first showed that the src oncogene of Rous Sarcoma virus derives from a normal cellular sequence (1). We now know that this normal precursor, the c-src protooncogene, is only one of a large array of genes that reside in the normal genome and can induce malignant phenotypes upon appropriate activation. Because of these properties, the protooncogenes now loom as centrally important elements in the molecular steps leading to human neoplasias.

Much effort has been spent identifying and cataloging these genes. It was retroviruses like Rous Sarcoma virus that provided us with the largest list of these genes. We now know of a group array of acutely transforming retroviruses, each of which, like the Rous prototype, has been found to carry an oncogene whose origins can be traced back to a precursor residing in the cellular genome (2).

A second line of experiments has utilized the procedures of gene transfer (transfection) to define transforming genes that are present in tumors bearing no apparent retrovirus genomes. Such transfections have defined a relatively small repertoire of distinct oncogenes. Each of these arises from a normal cellular protooncogene by somatic mutational processes. Most of these transfected oncogenes have been found to be closely related to oncogenes activated in animals by retroviruses. Examples of these are the H-, N- and K-ras oncogenes often detected in human tumor DNAs by transfection (3).

Yet other oncogenes are being defined by karyologic analysis of human and rodent tumor cells and by molecular hybridization using probes derived from the repertoire of retrovirus-associated genes. Thus, chromosomal translocations often lead to juxtaposition of these protooncogenes with previously unlinked segments of the cellular genome. This leads in turn to deregulation of the protooncogenes or to synthesis of structurally abnormal proteins. Protooncogenes known from retrovirology (e.g. myc, abl) are often involved but not always - several novel protooncogenes have been discovered by analysis of translocations. Gene amplification is yet another commonly observed karyotypic abnormality. Sequence hybridization has led to the discovery of several novel myc-related genes in human neuroblastomas and small cell carcinomas (4).

In animals, retroviruses lacking acquired oncogenes are able to activate protooncogenes by integrating proviruses adjacent to these genes in the host cell chromosome. Once again, the myc gene, known originally from its presence as a transduced gene in the avian myelocytomatosis genome, is often involved. But novel protooncogenes, activated by provirus insertion in mouse mammary carcinomas (int-1,2) have also been uncovered through this route (2-4).

Having uncovered these genes and deciphered their structure by nucleotide sequence analysis, we are now confronted by a far more daunting task - that of determining the mechanisms by which the oncogene proteins are able to perturb cellular phenotype. Indeed, the activities of these proteins must be viewed in two contexts - in that of the oncogene-transformed cell and in the normal cell where these proteins play apparently central regulatory roles.

The clues we have concerning function are still relatively minimal. The primary structures of virtually all the oncogene proteins are known through nucleotide sequencing of the oncogenes themselves. This information allows grouping these proteins, as is done here, by homology. Besides this information, we know that one

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group of proteins includes those that act enzymatically as tyrosine kinases while a second group (ras) includes those that bind and hydrolyze GTP (4).

To date this scant information has allowed little insight into the biochemical mechanism of action. While a number of substrates of the kinases have been catalogued, the relevance of any one of these to induction of transformation is obscure. The proteins of the ras group presumably act as regulatory subunits to a catalytic protein that also eluded identification.

Perhaps most intriguing are the roles of the proteins in normal cell physiology. Some undoubtedly mediate normally regulated cell growth, malignant deregulation being only a caricature of these normal processes. Others may be involved, unexpectedly, in quite unrelated activities. The high concentrations of the src protein in non-dividing cells of the central nervous sytem suggests a role unrelated to regulation of mitogenesis. While the attached collection of abstracts is extensive, the papers represented by them only begin to provide clues to these puzzles.

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1. Stehelin, D., Varmus, H.E., Bishop, J.M., and Vogt, P.K. (1976) DNA Related to the Transforming Gene(s) of Avian Sarcoma Viruses is Present in Normal Avian DNA. Nature 260: 170-173.
2. Varmus, H.E. (1984) The Molecule Genetics of Cellular Oncogenes. Ann. Rev. of Genetics 18: 553-612.
3. Cooper, G.M. (1982) Cellular Transforming Genes. Science 217: 801-806.
4. Weinberg, R.A. (1985) Action of Oncogenes in the Cytoplasm and Nucleus. Science 230: 770-776.

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ABSTRACTS

I.

PROTEINS OF RETROVIRAL AND CELLULAR ONCOGENES

A.

CYTOPLASMIC PROTEIN PRODUCTS OF CELLULAR ONCOGENES: PROTEIN KINASE ACTIVITY

1.

src protein product

1. HUMAN CELLULAR SRC GENE: NUCLEOTIDE SEQUENCE AND DERIVED AMINO ACID SEQUENCE OF THE REGION CODING FOR THE CARBOXY-TERMINAL TWO-THIRDS OF PP60C-SRC

Anderson SK. Gibbs CP. Tanaka A. Kung HJ. Fujita DJ
Cancer Research Laboratory. University of Western Ontario. London, Canada.
Mol Cell Biol. 5(5):1122-9 1985

The nucleotide sequence of the 3' two-thirds of a highly conserved, molecularly cloned human cellular src gene (c-src) has been determined. This region of the c-src gene encodes the tyrosine kinase domain of the cellular src protein (pp60c-src) and corresponds to exons 6 through 12 of the chicken c-src gene, as well as nucleotides 545 to 1542 of the Rous sarcoma virus src gene (v-src). The human c -src sequence is very strongly conserved with respect to both the chicken c-src and the Rous sarcoma virus v-src genes, with nearly 90% nucleotide homology observed in this region. Amino acid sequence conservation in this region is even greater; 98% of the amino acids are conserved between human and chicken c-src. Furthermore, the exon sizes and the locations of the exon-intron boundaries are identical in the human and chicken c-src genes. However, sequences within the introns have not been conserved, and the introns within the human c-src gene are significantly larger than the corresponding introns within the chicken c-src gene. The strong amino acid conservation between the carboxy-terminal two-thirds of pp60c-src of species as divergent as humans and chickens suggests that this portion of the pp60c-src protein specifies one or more functional domains that are of great importance to some aspect of normal cellular growth or differentiation.

2. ROUS SARCOMA VIRUS VARIANTS THAT ENCODE SRC PROTEINS WITH AN ALTERED CARBOXY TERMINUS ARE DEFECTIVE FOR CELLULAR TRANSFORMATION

Wilkerson VW. Bryant DL. Parsons JT
Department of Microbiology. University of Virginia School of Medicine 22908.
J Virol. 55(2):314-21 1985

The src gene of Rous sarcoma virus (v-src) and its cellular homolog, the c-src gene, share extensive sequence homology. The most notable differences between these genes reside in the region encoding the carboxy terminus of the src proteins. We constructed mutations within the 3' end of the v-src gene to determine the significance of this region to the transforming potential of the v-src protein, pp60v-src. The mutants CHdl300 and CHis1511 contain mutations that alter the last 23 amino acids of pp60v-src, whereas the mutant CHis1545-C contains a linker insertion that alters the last 11 amino acids of pp60v-src, and the mutant CHis1545-H contains a linker insertion that results in a 9-amino-acid insertion at position 415. Plasmids bearing each of these mutations were unable to transform chicken cells when introduced into these cells by DNA transfection. In addition, the structurally altered src proteins encoded by the mutants had much-reduced levels of tyrosine protein kinase activity in vivo, as measured by autophosphorylation and phosphorylation of the 34,000-Mr cellular protein, and in vitro, as determined by measuring the level of pp60src autophosphorylation. These data indicate that the carboxy-terminal amino acid sequences play an important role in maintaining the structure of the catalytic domain of pp60v-src. In contrast, the transfection of chicken cells with plasmid DNA containing a chimeric v-c-src gene resulted in morphological cell transformation and the synthesis of an enzymatically active hybrid protein. Therefore, the carboxy-terminal sequence alterations observed in the c-src protein do not alone serve to alter the functional activity of a hybrid v-c-src protein appreciably.

3. EXPRESSION OF V-SRC AND CHICKEN C-SRC IN RAT CELLS DEMONSTRATES QUALITATIVE DIFFERENCES BETWEEN PP60V-SRC AND PP60C-SRC

Parker RC. Varmus HE. Bishop JM
Department of Microbiology and Immunology. University of California, San Francisco.
Cell. 37(1):131-9 1984

The retroviral oncogene v-src arose by transduction of the cellular gene c-src. The similarity between these genes raised the possibility that c-src might be able to elicit neoplastic growth. We explored this by constructing a chimeric plasmid that allows the expression of chicken c-src. A rat cell line containing ten times the normal intracellular level of pp60c-src was isolated after transfecting rat-2 cells with the chimeric DNA. These cells produce the protein encoded by c-src (pp60c -src) in quantities at least three times greater than required to achieve transformation by the product of v-src (pp60v-src). The cells remain phenotypically normal, contain actin cables, and do not grow in soft agar. However, transfection of the cell line containing elevated cells of pp60c -src or Rat-2 cells with a molecular clone of v-src produces cells that exhibit properties of biologically transformed cells: round morphology, disrupted actin cables, and ability to grow in soft agar.

4. FUNCTIONAL SIMILARITIES AND DIFFERENCES BETWEEN VIRAL AND CELLULAR ONCOGENES (MEETING ABSTRACT)

Hanafusa H. Iha H. Cross F. Takeya T. Hanafusa T. Garber E. Pellman D
The Rockefeller Univ.
Genetics, Cell Differentiation, and Cancer. Seventh Annual Bristol-Myers Symposium on Cancer Research. October 8-9, 1984. Memorial Sloan-Kettering Cancer Center, p. 19-20, 1984.

Transfection of chicken cells with DNA of Rous sarcoma virus (RSV) results in the formation of infectious virus and cell transformation. This system was used to examine wheth-

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er the cellular src gene is capable of inducing cell transformation when its expression is elevated. The replacement of the first two-thirds or the remaining one-third of the v-src sequence with the corresponding c-src sequence does not affect the transforming activity of the resultant virus, indicating that v-src has at least two mutations critical for transformation. On the other hand, when the v-src sequence is totally replaced by the c-src sequence, we found the product p69c-src is overexpressed without appreciable transformation. The c-src-containing virus, however, mutates spontaneously to transforming virus at a relatively high frequency. The ability of various viruses including spontaneous mutants to cause cell transformation correlates well with the level of tyrosine-specific protein kinase activity of p60src they produce. Therefore, unlike many other oncogenes, mutation(s) in addition to transcriptional activation are required to convert the c-src proto-oncogene to the v-src oncogene. Analysis of in vitro constructed mutants of RSV revealed other aspects of transforming activity of p60src. In addition to the protein kinase activity, the capacity of p60src to associate with the plasma membrane in infected cells is critical for cell transformation. Particularly the amino-terminal fourteen amino acids are required for the attachment of myristic acid to p60src, for membrane association and for cell transformation. We speculate that the fatty acid attachment on p60src is required for its cell membrane association, which in turn is required for cell transformation.

5. THE PROTEIN ENCODED BY THE TRANSFORMING GENE OF AVIAN SARCOMA VIRUS (PP60SRC) AND A HOMOLOGOUS PROTEIN IN NORMAL CELLS (PP60PROTO-SRC) ARE ASSOCIATED WITH THE PLASMA MEMBRANE

Courtneidge SA, Levinson AD, Bishop JM
Dept. Microbiology and Immunology, Univ. California, San Francisco, CA 94143
Proc. Natl. Acad. Sci. U.S.A. 77:7 (3783-3787) 1980

Oncogenesis by avian sarcoma virus is attributable to a single viral gene (src) which encodes a phosphoprotein (pp60src) with the enzymatic activity of protein kinase. A closely related protein, pp60proto-src, occurs in uninfected cells from a wide variety of vertebrate species and is presumed to be the product of a cellular gene that served as progenitor for src. We explored the location of these proteins within the cell by using immunoprecipitation to analyze subcellular fractions prepared from avian sarcoma virus-transformed rat and chicken cells and from uninfected rat cells. We found that both pr60src and pp60proto-src were associated with the plasma membrane as active protein kinases and could be recovered efficiently only by disrupting the membranes with nonionic detergent. Our findings, in conjunction with those of other investigators, indicate that both proteins are embedded in the membrane by means of a hydrophobic domain(s); available evidence indicates that pp60src is not exposed on the surface of the cell but is accessible at the cytoplasmic aspect of the plasma membrane. These conclusions lend credence to two current speculations. First, pp60src and pp60proto-src may have similar or even identical functions. Second, neoplastic transformation may originate from derangements in the plasma membrane or its affiliated structures. (Author abstract) (28 Refs)

6. TYR527 IS PHOSPHORYLATED IN PP60C-SRC: IMPLICATIONS FOR REGULATION

Cooper JA, Gould KL, Cartwright CA, Hunter T
Fred Hutchinson Cancer Research Center, Seattle, WA 98104
Science 231:4744 (1431-4) 1986

The Rous sarcoma virus oncogene product, pp60v-src, transforms cultured fibroblasts but its corresponding proto-oncogene product, pp60c-src, does not. Both proteins are known to be protein-tyrosine kinases. Published results suggest that the kinase activity of pp60c-src is inhibited relative to that of pp60v-src, due perhaps to phosphorylation of a tyrosine in pp60c-src that is not phosphorylated in pp60v-src. In this study, it was observed that the tyrosine phosphorylated in pp60c-src is Tyr527 , six residues from the COOH-terminus of the protein. The region of pp60c-src from residue 515 to the COOH-terminus, including Tyr527, has been replaced with a different sequence in pp60v-src. Thus, the increase in transforming ability and kinase activity that occurred in the genesis of pp60v-src may have resulted from the loss of a tyrosine involved in negative regulation.

7. PHOSPHORYLATION OF TYROSINE IN THE CARBOXYL-TERMINAL TRYPTIC PEPTIDE OF PP60C-SRC

Lasudrey AP, Buchanan JM
Department of Biology, Massachusetts Institute of Technology, Cambridge 02139
Proc. Natl. Acad. Sci. U.S.A. 83:4 (802-6) 1986

The major site of tyrosine phosphorylation of the transforming protein of Rous sarcoma virus, pp60v-src (tyrosine-416), is different from the major site of tyrosine phosphorylation of its nontransforming normal cellular counterpart, pp60c-src. We have shown that antibodies against a synthetic peptide modeled on the carboxyl-terminal 13 residues of pp60c-src specifically immunoprecipitate the major phosphotyrosine tryptic peptide of pp60c-src from both chicken and rat fibroblasts. These experiments localize the major site of tyrosine phosphorylation to one or more of the three tyrosine residues in the carboxyl-terminal tryptic peptide at positions 511, 519, and 527 of the amino acid sequence of chicken pp60c-src. Tyrosines-519 and -527 are in the carboxyl-terminal 19-amino acid segment of pp60c-src that is deleted and replaced by an unrelated sequence in pp60v-src. It is possible that phosphorylation of tyrosine in the carboxyl-terminal tryptic peptide may be involved in the normal regulation of pp60c-src. The absence of this phosphorylation site in pp60v-src may, in part, contribute to its oncogenic properties.

8. BLOOD PLATELETS EXPRESS HIGH LEVELS OF THE PP60C-SRC-SPECIFIC TYROSINE KINASE ACTIVITY

Golden A, Nemeth SP, Brugge JS
Department of Microbiology, State University of New York, Stony Brook 11794
Proc. Natl. Acad. Sci. U.S.A. 83:4 (852-6) 1986

We have examined human and rabbit blood platelets for expression of pp60c-src, the normal cellular homolog of the transforming protein of Rous sarcoma virus. pp60c-src kinase activity was determined by an immune-complex kinase assay that uses enolase as the substrate, and pp60c-src protein levels were determined by an immunoblot assay. Lysates from platelets expressed high levels of pp60c-src-specific kinase activity and pp60c-src protein compared to the levels found in other tissues. pp60c-src was also found to be one of the major proteins phosphorylated in vitro in membranes isolated from platelets. Multiple protein species other than pp60c-src were also phosphorylated on tyrosine in the membrane phosphorylation reactions, and phosphotyrosine represented approximately equal to 80% of the total phosphoamino acid residues phosphorylated in the membranes. These results indicate that tyrosine kinases represent the major protein phosphorylating enzymes detected in isolated platelet membranes. Although the association of tyrosine kinase activity with many viral oncogene products and cellular growth hormone receptors has suggested a role for these enzymes in the regulation of cell proliferation, these results indicate that

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the expression of high levels of tyrosine kinase activity is not exclusively associated with proliferating cells.

9. INCREASED PP60C-SRC TYROSYL KINASE ACTIVITY IN HUMAN NEUROBLASTOMAS IS ASSOCIATED WITH AMINO-TERMINAL TYROSINE PHOSPHORYLATION OF THE SRC GENE PRODUCT

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Proc Natl Acad Sci USA; 82(21):7275-9 1985

We have observed a 20- to 40-fold increase in pp60c-src tyrosyl kinase activity in human neuroblastoma cell lines over that found in either human glioblastoma cells or human fibroblasts. The level of c-src gene transcripts and pp60c-src protein synthesis in the neuroblastoma cells was not significantly increased when compared to the levels found in glioblastoma cells. Approximately one half of the pp60c-src molecules synthesized during a 4-hr [35S]methionine or [32P]orthophosphate labeling period in neuroblastoma cells were found to migrate more slowly on NaDodSO4/polyacrylamide gels than pp60c-src molecules labeled in glioblastoma cells. Peptide and phosphoamino acid analysis of the in vivo phosphorylated c-src molecules from these two cell types revealed that pp60c-src molecules from the neuroblastoma cells possess in the amino-terminal portion of the protein at least one unique tyrosine phosphorylation site not found in pp60c-src derived from glioblastoma cells.

10. ANALYSIS OF PP60C-SRC PROTEIN KINASE ACTIVITY IN HAMSTER EMBRYO CELLS TRANSFORMED BY SIMIAN VIRUS 40, HUMAN ADENOVIRUSES, AND BOVINE PAPILLOMAVIRUS 1

Amini S, Lauts AM Jr, Israel MA, Butel JS, Bolen JB
Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, Maryland 20205
J Virol; 57(1):357-61 1986

We have examined the effect of DNA tumor virus transformation of primary hamster embryo cells on the tyrosyl kinase activity of pp60c-src. Our present study demonstrates that some clones of hamster embryo cells transformed by simian virus 40, adenovirus type 2, adenovirus type 12, or bovine papillomavirus 1 can possess elevated pp60c-src kinase activity when compared with normal hamster embryo cells. However, other clones of hamster embryo cells transformed by these same viruses were found to have normal levels of pp60c-src kinase activity. In those clones of transformed cells where pp60c-src kinase activity was elevated, the increased levels of kinase activity were the result of an apparent increase in the specific activity of the pp60c-src phosphotransferase rather than an increase in the amount of the src gene product. Additionally, pp60c-src was not found to be physically associated with tumor antigens known to be encoded by these viruses. These results indicate that elevated levels of pp60c-src kinase activity can be found in hamster embryo cells transformed by several different DNA tumor viruses and suggest that the molecular mechanism by which pp60c-src kinase activity is elevated may differ from that previously observed in polyomavirus-transformed cells. These results also imply that elevation of pp60c-src kinase activity is not required for the transformation of hamster cells by these viruses.

11. A MUTATION AT THE ATP-BINDING SITE OF PP60V-SRC ABOLISHES KINASE ACTIVITY, TRANSFORMATION, AND TUMORIGENICITY

Snyder MA, Bishop JM, McGrath JP, Levinson AD
G. W. Hooper Foundation, San Francisco, California 94143
Mol Cell Biol; 5(7):1772-9 1985

We constructed a mutant, called RSV-SF2, at the ATP-binding site of pp60v-src. In this mutant, lysine-295 is replaced with methionine. SF2 pp60v-src was found to have a half-life similar to that of wild-type pp60v-src and was localized in the membranous fraction of the cell. Rat cells expressing SF2 pp60v-src were morphologically untransformed and do not form tumors. The SF2 pp60v-src isolated from these cells lacked kinase activity with either specific immunoglobulin or other substrates, and expression of SF2 pp60v-src failed to cause an increase of total phosphotyrosine in the proteins of infected cells. Wild-type pp60v-src was phosphorylated on serine and tyrosine in infected cells, and the analogous phosphorylations could also be carried out in vitro. Phosphorylation of serine was catalyzed by a cyclic AMP-dependent protein kinase, and phosphorylation of tyrosine was perhaps catalyzed by pp60v-src itself. By contrast, SF2 pp60v-src could not be phosphorylated on serine or tyrosine either in infected cells or in vitro. These findings strengthen the belief that the phosphotransferase activity of pp60v-src is required for neoplastic transformation by the protein and suggest that the binding of ATP to pp60v-src elicits an allosteric change required for phosphorylation of serine in the protein.

12. DIRECT EVIDENCE THAT ONCOGENIC TYROSINE KINASES AND CYCLIC AMP-DEPENDENT PROTEIN KINASE HAVE HOMOLOGOUS ATP-BINDING SITES

Kamps MP, Taylor SS, Sefton BM
Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California
Nature; 310(5978):589-92 1984

p60src, the transforming protein of Rous sarcoma virus (RSV), is a protein kinase that has a strict specificity for tyrosine. The phosphorylation of cellular proteins by p60src (ref. 4) results in transformation. Recently, Barker and Dayhoff discovered that residues 259-485 of p60src have 22% sequence identity with residues 33-258 of the catalytic subunit of cyclic AMP-dependent protein kinase, an enzyme that has a specificity for serine. Because it was necessary to introduce eight gaps to align the two proteins, the question remained as to whether this apparent homology reflected a common evolutionary origin. We demonstrate here that the ATP analogue p-fluorosulphonylbenzoyl 5'-adenosine (FSBA) inactivates the tyrosine protein kinase activity of p60src by reacting with lysine 295. When aligned for maximum sequence identity, lysine 295 of p60src and the lysine in the catalytic subunit which also reacts specifically with FSBA are superimposed precisely. This functional homology is strong evidence that the protein kinases, irrespective of amino acid substrate specificity, comprise a single divergent gene family.

13. PARTIAL PURIFICATION AND CHARACTERIZATION OF A PP60V-SRC-RELATED TYROSINE KINASE FROM BOVINE BRAIN

Neer EJ, Lok JM
Department of Medicine, Brigham and Women's Hospital, Boston, MA
Proc Natl Acad Sci USA; 82(18):6025-9 1985

We have identified and substantially purified a tyrosine protein kinase from normal bovine brain that is immunologically related to the product of the Rous sarcoma virus oncogene (pp60v-src). The enzyme, a 61-kDa protein (p61), is solubilized with detergent from bovine cerebral cortical mem-

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branes and purified by column chromatography. In the purest preparations, this protein is phosphorylated only on tyrosine, but it can also be a substrate for serine- and threonine-specific protein kinases. The p61 protein phosphorylates the heavy chain of immunoglobulins from rabbits bearing Rous sarcoma virus-induced tumors (TBR IgG) but not normal IgG. TBR IgG precipitates the 61 kDa phosphoprotein and protein kinase activity from purified preparations. The activity of the purified brain tyrosine kinase is 10 times higher in the presence of 7-10 mM Mn2+ and 6 mM Mg2+ than it is with 6 mM Mg2+ alone. With Mn2+, the p61 enzyme has a Km for ATP of 2 microM. All preparations of p61 also contain a 64-kDa protein (p64) that is phosphorylated on tyrosine. Measurement of the Stokes radius of p61 and p64 by gel filtration shows that they are not physically associated in buffer containing the nonionic detergent Lubrol 12A9. The p64 protein is not precipitated by TBR IgG. We do not know whether p64 is only a substrate for the p61 tyrosine kinase or is itself a kinase.

14. STUDY OF PP60V-SRC PROTEIN KINASE ACTIVITY IN SYNCHRONIZED CHICKEN EMBRYO FIBROBLASTS INFECTED WITH ROUS SARCOMA VIRUS

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Exp Cell Res. 150(1):242-9 1984

Chicken embryo fibroblast (CEF) cultures, synchronized by the addition of serum to stationary cells, were exposed to Schmidt-Ruppin strain of Rous Sarcoma Virus (SR-RSV) and the appearance of pp60v-src protein kinase activity was examined through the cell cycle. In cells infected either at the beginning or at the end of G1, the onset of pp60v-src protein kinase activity was coincidental, closely following mitosis, with a delay between the infection of cells with SR-RSV and the appearance of protein kinase activity of about 20 and 16 h, respectively. In cells infected during the S phase this delay was 16 h, as observed for late G1 cells. These experiments show that the activity of pp60v-src protein kinase, which cannot be detected before the first mitosis following infection does not depend on G1. The aphidicolin prevented protein kinase activity if added before or at the beginning of S phase, but not if added later, which is presumably related to the inhibition of S phase, required for provirus integration. The use of colcemid, which suppresses cell division, did not inhibit but delayed the appearance of protein kinase activity. These results show that the synthesis of an active oncogene product, such as pp60v-src protein kinase, depends on both S phase and mitosis.

15. PHOSPHORYLATION OF TYROSINE-416 IS NOT REQUIRED FOR THE TRANSFORMING PROPERTIES AND KINASE ACTIVITY OF PP60V-SRC

Snyder MA. Bishop JM. Colby WW. Levinson AD

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Cell. 32(3):891-901 1983

A mutant in src, the oncogene of Rous sarcoma virus, has been constructed in which the major phosphorylated tyrosine (Tyr-416, located in the carboxy-terminal half of the protein) has been replaced by phenylalanine. Mouse cells transformed with this mutant src form foci and grow in soft agar, indicative of a transformed state. Also, the mutant protein retains the wild-type ability to phosphorylate proteins on tyrosine. Partial proteolysis revealed that the carboxy-terminal half of the mutant protein was still phosphorylated, although apparently to a lesser extent. Analysis indicated that this residual phosphorylation was on tyrosine. We conclude that the major tyrosine phosphorylation in pp60v-src is not required for two of the protein's notable properties-protein kinase activity and transformation of cultured cells. (Author abstract) (51 Refs)

16. THE EXPRESSION IN EUKARYOTES OF A TYROSINE KINASE WHICH IS REACTIVE WITH PP60V-SRC ANTIBODIES

Schartl M. Barnekow A

Genetisches Institut der Justus-Liebig-Universitat Giessen, Heinrich-Buff-Ring 58-62, D-6300 Giessen, W. Germany

Differentiation. 23(2):105-114 1982

All specimens of Eumetazoa and Parazoa, ranging from mammals, birds, teleosts, sharks, lampreys, amphioxus, insects, down to sponges showed the pp60c-src associated kinase activity, indicating that c-src, which is the cellular homologue of the oncogene v-src of Rous sarcoma virus (RSV) is probably present in all multicellular animals. Protozoa and plants did not show pp60c-src kinase activity. The degree of c-src expression depends on the taxonomic rank of the Eumetazoa tested, and is organ-specific with nervous tissues displaying the highest kinase activities. In the central nervous system of mammals and birds we found a high c-src expression, and in that of the lampreys, amphioxus, and insects the lowest. Unexpectedly, total extracts of sponges showed an amount of pp60c-src kinase activity similar to that of brain cell extracts of mammals and birds. These findings suggest that pp60c-src is a phylogenetic old protein that might have evolved together with the multicellular organization of Metazoa, and that might be of importance in proliferation and differentiation of nontransformed cells. (Author abstract) (40 Refs)

17. MATERNAL INHERITANCE OF TRANSCRIPTS FROM THREE DROSOPHILA SRC-RELATED GENES

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Nucleic Acids Res. 13(6):2153-70 1985

The Drosophila genome contains three major sequences related to the v-src gene. Previously published molecular studies have confirmed the structural homology between v-src and two of the Drosophila sequences. We have sequenced a portion of the third v-src-related Drosophila gene and found that it also shares structural homology with vertebrate and Drosophila src-family genes. RNA sequences from each of the src genes are present in pre-blastoderm embryos indicating that they are of maternal origin. As embryogenesis proceeds, the levels of each of the src RNA sequences decline. The pre-blastoderm src gene transcripts contain poly(A) and are present on polyribosomes suggesting that they are functional mRNAs. Since the Drosophila src transcripts were maternally inherited, we also investigated their distribution in adult females. The majority of the src transcripts in adult females were contained in ovaries. Only low levels of the transcripts were detected in males. These results strongly suggest that an abundant supply of src protein is required during early embryogenesis, perhaps at the time of cellularization of the blastoderm nuclei.

18. THE NUCLEOTIDE SEQUENCE AND THE TISSUE-SPECIFIC EXPRESSION OF DROSOPHILA C-SRC

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Cell. 42(3):831-40 1985

We have examined the coding capability and expression of the Drosophila homolog of the vertebrate proto-oncogene c-src. Sequence analysis of a cDNA clone representing the

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Drosophila c-src locus suggests that the gene encodes a 62 kd protein that is remarkably similar to the protein product of chicken c-src. The Drosophila c-src locus is transcribed into three mRNAs that are each regulated independently during development. Drosophila c-src RNA is abundant in embryos and pupae but rare in larvae and adults. In situ hybridization reveals that after the first 8 hr of development, c-src RNA accumulates almost exclusively in neural tissues such as the brain, ventral nerve chord, and eye-antennal discs, and in differentiating smooth muscle. We conclude that c-src may not be a mitotic signal but instead may play a role in the development of neural tissue and smooth muscle.

19. THREE LOCI RELATED TO THE SRC ONCOGENE AND TYROSINE-SPECIFIC PROTEIN KINASE ACTIVITY IN DROSOPHILA

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Nature; 302(5911):837-9 1983

Rous sarcoma virus (RSV) is an acutely oncogenic avian retrovirus which induces sarcomas in animals and transforms fibroblasts in cell culture. Genetic analysis indicates that the viral src gene (v-src) mediates neoplastic transformation. The product of v-src is a 60,000 molecular weight (MW) phosphoprotein (pp60v-src) possessing the enzymatic activity of a tyrosine-specific protein kinase. The viral src gene is derived from a cellular gene (c-src) which also encodes a 60,000 MW phosphoprotein (pp60c-src) with tyrosine-specific protein kinase activity. Both birds and mammals are known to possess c-src. Shilo and Weinberg have reported that the genome of the fruit fly, Drosophila melanogaster, contains nucleotide sequences that are homologous to v-src. We report here the molecular cloning and chromosomal mapping of three loci from the Drosophila genome that contain such sequences. We also show that Drosophila contain both phosphotyrosine and a tyrosine-specific protein kinase activity immunoprecipitated by antisera directed against pp60v-src. It should now be possible to identify the precise locus that encodes a src-specific protein kinase in Drosophila, and to explore the role of c-src in the growth and development of D. melanogaster.

20. REGULATORY PROTEINS OF THE CYTOSKELETON SYSTEM AND THEIR CHANGES ASSOCIATED WITH CARCINOGENESIS

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Gan To Kagaku Ryoho; 10(2 Pt 2):527-31 1983

Microfilaments are known to participate in several cellular functions. Proteins which cross-link, sever or cap F-actin filaments, and those inhibit polymerization of G-actin may regulate intracellular distribution, length and amount of F-actin to cope with wide varieties of cellular functions of microfilaments. Actinogelin, a calcium-regulated crosslinking protein of F-actin, was discovered in and purified from Ehrlich tumor cells. Native molecule of the protein was found to be consisted of two 110,000-115,000 dalton subunits. Gelation of F-actin can be induced by the addition of the purified protein, and this can be inhibited by Ca2+ (half maximal inhibition, 2 microM). Actinogelin is distributed in several types of cells including fibroblasts, epithelial cells, macrophages and lymphocytes. This protein is found to localize at crossing or converging points of stress fibers (bundle of microfilaments) by immunofluorescence staining using anti-actinogelin antibody. Adhesion plaques of fibroblasts are also stained. Since oncogene product of Rous sarcoma virus, pp 60src, is also present in adhesion plaques, possible phosphorylation of actinogelin in a RSV-transformed cells was studied by the immunoblotting technique. It was found that phosphorylation of actinogelin occurred only at permissive temperature. This modification of the protein might be a cause of disappearance of stress fibers from cancer cells.

21. SCREENING OF AGENTS WHICH CONVERT 'TRANSFORMED MORPHOLOGY' OF ROUS SARCOMA VIRUS-INFECTED RAT KIDNEY CELLS TO 'NORMAL MORPHOLOGY': IDENTIFICATION OF AN ACTIVE AGENT AS HERBIMYCIN AND ITS INHIBITION OF INTRACELLULAR SRC KINASE

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Institute of Microbial Chemistry, Tokyo.
Jpn J Cancer Res; 76(8):672-5 1985

During the course of screening of agents active in converting the transformed morphology of Rous sarcoma virus-infected rat kidney cells to the normal morphology, we identified an active substance produced by Streptomyces sp. MH237-CF8 as herbimycin. Herbimycin converted almost all cells into the normal morphology. The antibiotic was found to be an inhibitor of p60src-associated protein kinase in the cells.

22. SMALL DELETION IN SRC OF ROUS SARCOMA VIRUS MODIFYING TRANSFORMATION PHENOTYPES: IDENTIFICATION OF 207-NUCLEOTIDE DELETION AND ITS SMALLER PRODUCT WITH PROTEIN KINASE ACTIVITY

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J Virol; 46(3):985-92 1983

Partial deletion in the src gene and the gene product were characterized in a deletion mutant, dl5, isolated from the Prague strain of Rous sarcoma virus. The mutant induced fusiform-like transformed cells, unlike the parental Prague strain, which induced round transformed cells. Determination of the total nucleotide sequences of src in dl5 and the Prague strain of Rous sarcoma virus demonstrated that in the former two deletions of 196 and 11 nucleotides had occurred at positions 403 and 696, respectively, from the 5' end of src. A protein with a molecular weight of 52,000 (p52src) was detected in cells infected with dl5, as predicted from the deletion size in src. From the nucleotide sequence, it was predicted that p52src had two deletions of 65 and 4 amino acids at positions 135 and 232, respectively, from the N-terminal methionine of p60src and also had 33 amino acid changes between these two deletion sites due to alteration of the reading frame. p52src, which contained deletions and alterations of amino acids near the N-terminus, showed protein kinase activity similar to that of p60src and functioned in the infected cells. These results strongly suggest that changes in the N-terminal region of p60src modified its transforming ability, causing induction of the fusiform-like transformation phenotype.

23. SARC-SPECIFIC PROTEIN KINASE ACTIVITY IN RIBOSOME FRACTION OF CELLS TRANSFORMED WITH ROUS SARCOMA VIRUS

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N. N. Petrov Res. Inst. Oncology, Leningrad, USSR
Dokl Akad Nauk SSSR; 266(5):1277-1280 1982

Specific ribosome-associated activity of sarc-protein (the product of oncogene expression) was studied in Rous sarcoma cells. Analysis of the ribosome fraction showed the presence of specific protein kinase (PK) activity. Experiments with heat inactivation showed that PK in the cells transformed with Rous sarcoma virus was more than three times that in the normal cells. (14 Refs)

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24. PARTIAL CHARACTERIZATION OF THE MITOGENIC ACTION OF PP60V-SRC, THE ONCOGENIC PROTEIN PRODUCT OF THE SRC GENE OF AVIAN SARCOMA VIRUS

Durkin JP, Whitfield JF
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J Cell Physiol; 120(2):135-45 1984

NRK cells infected with a temperature-sensitive, transformation-defective mutant of avian sarcoma virus (ASV), tsLA23, are transformed at 36 degrees C, but at 40 degrees C they behave as nontransformed cells because of the inactivation of the abnormally thermolabile pp60v-src product of the virus' transforming src gene. At 40 degrees C, these tsLA23-NRK cells were arrested in G1/G0 by severe serum deprivation. They were induced to enter G1, initiate DNA synthesis 7 or 10 hours later, and then divide as (1) nontransformed cells by adding serum or platelet-derived growth factor (PDGF) at 40 degrees C, or (2) transformed cells by lowering the temperature to a pp60v-src-activating 36 degrees C without adding exogenous growth factor(s). The level of pp60v-src kinase activity rose dramatically in these serum-deprived cells within 30 minutes of lowering the temperature to the permissive 36 degrees C, and it fell just as rapidly when the cells were returned to the restrictive 40 degrees C. As little as a 2-hour exposure to 36 degrees C, with an attendant 2-hour burst of pp60v-src kinase activity, was enough to stimulate serum-deprived tsLA23-NRK cells to transit G1 and initiate DNA replication, but not to divide. Much more prolonged pp60v-src activity was needed for these serum-deprived cells to complete their cycle and divide. The prereplicative development of quiescent tsLA23-NRK cells stimulated by serum or PDGF was accompanied by greatly increased protein synthesis and slightly decreased protein degradation, but the pp60v-src-stimulated cells progressed through G1 and initiated DNA replication without appreciably affecting the protein synthetic machinery of the cell. The cells stimulated by the mitogenic action of pp60v-src, like the cells stimulated by serum, needed to activate early prereplicative genes in order to initiate DNA replication. The needed RNA transcripts induced by serum and pp60v-src were produced with comparable efficiency, although it took longer for pp60v-src-stimulated cells to translate these transcripts and to initiate DNA replication, probably because of their unstimulated protein-synthetic machinery.

25. NEOPLASTIC TRANSFORMATION OF NORMAL AND CARCINOGEN-INDUCED PRENEOPLASTIC SYRIAN HAMSTER EMBRYO CELLS BY THE V-SRC ONCOGENE

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Mol Cell Biol; 5(7):1707-13 1985

The ability of cloned Rous sarcoma virus (RSV) DNA encoding the v-src oncogene to neoplastically transform normal, diploid Syrian hamster embryo (SHE) cells was examined. Transfection of RSV DNA into early passage SHE cells resulted in a low but significant number of tumors when treated cells were injected into nude mice. Tumors formed with a low frequency (two tumors out of ten sites injected) and only after a long latency period (14 weeks). In contrast to the normal SHE cells, several different carcinogen-induced preneoplastic immortal SHE cell lines were highly susceptible to transformation by the v-src oncogene to the neoplastic phenotype. Tumors formed with high efficiency and a short latency period (less than 3 weeks). Further studies were performed to determine the basis for the inefficient transformation of the normal SHE cells. NeoR clones isolated after cotransfection of SHE cells with pSV2-neo and RSV DNAs were neither morphologically altered nor immortal and did not contain detectable levels of the v-src gene product. These results suggest that neoplastic transformation by v-src DNA in the normal cells is initially suppressed. However, cells from a v-src-induced tumor expressed v-src RNA, and antibody to v-src protein precipitated from the tumor cells a 60,000-molecular-weight protein which displayed protein kinase activity. Karyotypic analyses confirmed that the tumor was derived from Syrian hamster cells and suggested that it was clonal in nature. These results indicate that the v-src oncogene was primarily responsible for neoplastic transformation of SHE cells.(ABSTRACT TRUNCATED AT 250 WORDS)

26. DIFFERENTIATION OF PC12 PHAEOCHROMOCYTOMA CELLS INDUCED BY V-SRC ONCOGENE

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Nature; 316(6028):557-9 1985

PC12 rat phaeochromocytoma cells are a model system that can be used to study both neuronal differentiation and the mechanism of action of nerve growth factor (NGF). PC12 cells respond to NGF protein by shifting from a chromaffin-cell-like phenotype to a neurite-bearing sympathetic neurone-like phenotype. Here we present data on the effect of infection of PC12 cells with retroviruses carrying the src oncogene of Rous sarcoma virus. Previous studies have demonstrated that the expression of src severely affects the synthesis and accumulation of differentiated cell products in a variety of cell types. We show that in the PC12 cell system, expression of v-src appears to have an inductive effect on differentiation that resembles the action of a 'physiological' growth factor.

27. P60C-SRC ACTIVITY DETECTED IN THE CHROMAFFIN GRANULE MEMBRANE

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Biochem Biophys Res Commun; 134(2):736-42 1986

Using monoclonal antibodies specific for p60c-src we have detected high levels of this kinase in adrenal medullary chromaffin tissue and in highly purified chromaffin granule (secretory vesicle) membranes. An immune complex kinase assay was applied to fractions of adrenal medullary tissue resolved on sucrose density gradients. Thirty-seven per cent of the total tissue p60c-src activity was found in association with chromaffin granule or granule membrane markers. Localization of a significant fraction of total cellular p60c-src activity to this secretory vesicle membrane suggests that the kinase may function in the regulation of neurotransmitter release.

28. DIFFERENTIAL EXPRESSION OF THE CELLULAR ONCOGENES C-SRC AND C-YES IN EMBRYONAL AND ADULT CHICKEN TISSUES

Gessler M, Barnekow A
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Biosci Rep; 4(9):757-70 1984

The cellular onc-genes c-src and c-yes are expressed very differently during chicken embryonic development. The c-src mRNA and its translational product are detectable at high levels in brain extracts of chicken embryos and adult chickens, whereas muscle extracts show an age-dependent decrease in the amounts of c-src-specific mRNA and pp60c-src

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kinase activity. In contrast, the levels of c-yes mRNA in brain, heart, and muscle are relatively low in early embryonic stages and increase later on to values comparable to those found for liver, while in adult animals the pattern of c-yes expression is similar to that of the c-src gene. From the close correlation between the levels of pp60c-src, its enzymatic activity, and its corresponding mRNA at a given stage of development and in given tissues, it appears that the expression of pp60c-src is primarily controlled at the level of transcription. It is suggested that because of the different patterns of expression, the two cellular oncogenes, c-src and c-yes, play different roles in cell proliferation during early embryonic stages as well as in ensuing differentiation processes.

29. TRANSFORMATION OF ERYTHROID CELLS BY ROUS SARCOMA VIRUS (RSV)

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National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana
Virology, 140(2):269-80 1985

RSV transforms several nonhematopoietic cell types and as reported here also has the capacity to transform hematopoietic cells of the erythroid lineage. In vitro, the three RSV isolates tested induced erythroblast-like colonies in infected bone marrow cells that were distinguishable by size and cell arrangement from those induced by avian erythroblastosis virus (AEV). Also in contrast to AEV-transformed erythroblast cultures, isolated cell colonies induced by RSV required complex growth conditions in liquid medium similar to the in vitro conditions necessary for erythroblasts transformed by the acute leukemia virus E26. Temperature-shift experiments using temperature-sensitive (ts) NY68 RSV revealed that when grown at the nonpermissive temperature (42 degrees), mutant-infected cells became benzidine positive and partially differentiated into erythrocytes. Wild-type (wt) RSV-transformed cells did not undergo similar changes. However, both wt RSV-, and to a greater extent, ts RSV-transformed cultures at the permissive temperature (37 degrees) did contain populations of spontaneously differentiating erythroid cells signifying that the transforming activity of the virus did not fully arrest erythroid maturation. In addition, the RSV-transformed cells did express tyrosine kinase activity. When injected intravenously into birds, RSV induced an erythroblastosis-like disease similar to AEV but also caused fibrosarcomas and leg paralysis. These results show that RSV can alter the pattern of erythroid differentiation in a manner similar to, but distinct from, AEV and indicate that the tyrosine-specific pp60src kinase is involved in erythroid cell transformation. Since the src and erb B proteins share a significant amino acid homology, these data suggest that both may also share a common functional homology.

30. C-SRC IS CONSISTENTLY CONSERVED IN THE CHROMOSOMAL DELETION (20Q) OBSERVED IN MYELOID DISORDERS

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Proc Natl Acad Sci USA, 82(19):6692-6 1985

The proto-oncogene c-src has been mapped to two bands in human chromosomes, 1p36 and 20q13, both of which are involved in rearrangements in human tumors. In particular, deletions (loss of part of a chromosome) of the long arm of chromosome 20, del(20q), are commonly observed in hematologic malignant diseases. By using in situ chromosomal hybridization of a c-src probe to metaphase cells prepared from leukemic bone marrow cells of three patients with a del(20q), we observed specific labeling on the deleted chromosome in each patient, indicating that the c-src locus was conserved. The presence on the rearranged chromosomes of c-src, which is normally located on the most distal band of 20q, indicated that the deletions were not terminal as they appeared to be on the basis of chromosome morphology, but rather that they were interstitial. The location of c-src relative to the distal breakpoint in these deletions is unknown. By using the v-src probe in Southern blot analysis of genomic DNA from three patients with a del(20q), we found that no major genomic rearrangements or amplification of the c-src genes had occurred within the regions homologous to v-src. Our observation that c-src is consistently preserved in these rearranged chromosomes suggests that this gene may play a role in the pathogenesis of some myeloid disorders.

31. POTENTIAL ROLE OF THE SRC GENE PRODUCT IN INHIBITION OF GAP-JUNCTIONAL COMMUNICATION IN NIH/3T3 CELLS

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Proc Natl Acad Sci USA, 82(16):5360-4 1985

The effects of the src gene on the activity of protein kinase C and intercellular communication have been studied in transformed NIH/3T3 clones isolated from soft agar following transfection with the plasmid carrying the v-src gene (psrc-11). Six transformed clones that were studied contained newly incorporated v-src genes in the genome, had an increased amount of pp60src, and showed enhanced activities of protein kinase C. Intercellular communication, studied by observing with autoradiography the transfer of [3H]uridine nucleotide from prelabeled donor cells to recipient cells in contact, was found to be reduced in transformed clones as compared to parental NIH/3T3 cells. Treatment with phorbol 12-myristate 13-acetate was also found to increase protein kinase C activity and to reduce intercellular communication in normal NIH/3T3 cells. These results suggest that the v-src gene product, in a manner similar to some of the powerful tumor promoters, may directly or indirectly affect cell-cell communication.

32. A CELLULAR PROTEIN THAT ASSOCIATES WITH THE TRANSFORMING PROTEIN OF ROUS SARCOMA VIRUS IS ALSO A HEAT-SHOCK PROTEIN

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Proc Natl Acad Sci USA, 78(2,part2):1067-1071 1981

A single viral protein (pp60src) mediates neoplastic transformation of cells infected with Rous sarcoma virus. Immunoprecipitation of pp60src has revealed two cellular proteins (Mr 50,000 and 89,000) that appear to associate with pp60src in a specific manner. Neither of the cellular proteins has been well characterized, but it is thought that both may participate in the function of pp60src. Treatment of avian cells with unphysiological temperature or certain chemical agents amplifies the production of several proteins in the manner of the 'heat shock' response earlier described for Drosophila. We report here that one of these proteins, with a mol wt of 89,000, is identical to the 89-kilodalton protein found associated with pp60src. The 89-kilodalton protein is a major constituent of both uninfected and infected cells, even in the absence of inducing agents, but only a small fraction of this protein appears to associate with pp60src in cells transformed by Rous sarcoma virus. The complex containing pp60src and the 89-kilodalton protein can be precipitated by immune reaction involving pp60src alone. The complexed form of the 89-kilodalton protein did not react directly with antibodies but regained its reactivity subsequent to release from the complex. We conclude that the 89-kilodalton protein is

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bound to pp60src in a relatively stable complex. We suggest that the 89-kilodalton protein may have overlapping roles in viral oncogenesis and the heat shock response, and that evidence on the function of the protein in either setting may illuminate its function in the other. In addition, it may prove profitable to search for other overlaps between the cellular response to heat shock and the neoplastic transformation of cells by pp60src. (Author abstract) (22 Refs)

33. ONCOGENES

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Sci Am; 246(3):80-84,87-92,154 1982

Recent developments in cancer research concerning inducement of cancerous growth are reviewed. Some tumor viruses (TV) are oncogenic only in animals that are not hosts in nature, others are oncogenic only in their natural host. Transformation of cultured cells by TV makes possible the examination of the interaction of a TV with a host cell under controlled conditions, thus avoiding the difficulties associated with experiments in animals. Some viruses have a single gene which can induce tumors, or in some cases, a few such 'oncogenes'. Retroviruses (RV) are the only TV with an RNA genome. The src oncogene of Rous sarcoma virus is discussed. The protein encoded by src is a 60,000 mol wt phosphoprotein. Phosphorylation of tyrosine is a common characteristic of oncogene-encoded enzymes and also has a role in regulating the growth of normal cells. The mutational hypothesis proposes that viral oncogenes differ from their cellular progenitors as a result of mutations introduced when the cellular genes were copied into the RV genome. The dosage hypothesis suggests that RV oncogenes overburden cells with too much essentially normal proteins carrying out normal functions. Excessive activity might tip the balance of regulation toward incessant growth. In tumors induced by the chicken lymphoma viruses, the viral DNA is almost always inserted into cellular DNA in the immediate vicinity of a single cellular oncogene, and the expression of the cellular oncogene is greatly amplified. Gene-length bits of DNA isolated from some tumors can transmit the property of cancerous growth when they are introduced into previously normal cells in culture. (4 Refs)

34. IMMUNOPRECIPITATION OF INSULIN RECEPTORS FROM CULTURED HUMAN LYMPHOCYTES (IM-9 CELLS) BY ANTIBODIES TO PP60SRC

Perrotti N, Taylor SI, Richert ND, Rapp UR, Pastan IH, Roth J
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Science; 227(4698):761-3 1985

The family of tyrosine-specific protein kinases includes proteins encoded by retroviral oncogenes as well as receptors for insulin and several growth factors. Antibodies to pp60src, the protein encoded by the src oncogene of Rous sarcoma virus (RSV), can specifically immunoprecipitate affinity-labeled insulin receptors from cultured human lymphocytes (IM-9 cells). This precipitation is specifically inhibited by the src gene product purified from RSV-transformed rat cells. These observations provide evidence that there is structural homology between the insulin receptors and pp60src.

35. CHOLERA TOXIN TREATMENT STIMULATES TUMORIGENICITY OF ROUS SARCOMA VIRUS-TRANSFORMED CELLS

Gottesman MM, Roth C, Vlahakis G, Pastan I
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Mol Cell Biol; 4(12):2633-42 1984

Chinese hamster ovary cells transformed by Rous sarcoma virus form tumors poorly in nude mice. Tumorigenicity was markedly stimulated by pretreatment of the cells with cholera toxin, which raises cyclic AMP levels and activates cyclic AMP-dependent protein kinase. Increased tumorigenicity was manifested by a severalfold increase in the rate of tumor formation, as well as earlier appearance and more rapid growth of tumors. In contrast, spontaneously transformed Chinese hamster ovary cells showed decreased tumorigenicity after cholera toxin treatment. The activation of tumorigenic potential in Rous sarcoma virus-transformed Chinese hamster ovary cells by cholera toxin correlated with increased phosphorylation of the viral oncogene product pp60src and stimulation of its tyrosine kinase activity.

36. ENHANCEMENT OF CELLULAR SRC GENE PRODUCT ASSOCIATED TYROSYL KINASE ACTIVITY FOLLOWING POLYOMA VIRUS INFECTION AND TRANSFORMATION

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Cell; 38(3):767-77 1984

We examine the interaction between polyoma-virus-encoded middle tumor antigen and the cellular src gene product, pp60c-src, using a series of monoclonal antibodies that recognize mammalian pp60c-src. Our results show that infection of mouse cells with transformation-competent strains of polyoma virus results in the stimulation of pp60c-src kinase activity severalfold over that observed in uninfected mouse cells and mouse cells infected with transformation-deficient polyoma virus. A similar degree of enhancement of pp60c-src kinase activity was found in polyoma-virus-transformed rodent cells. No differences were detected in the level of pp60c-src synthesis in polyoma-virus-infected and uninfected mouse cells or polyoma-virus-transformed and normal rodent cells. These studies demonstrate that polyoma-virus-encoded middle tumor antigen is associated with pp60c-src in lysates of polyoma-virus-infected and polyoma-virus-transformed cells and suggest a novel mechanism for the functional activation of a cellular proto-oncogene product, namely, that the interaction between middle tumor antigen and pp60c-src leads to a stimulation of pp60c-src tyrosyl kinase activity.

37. COMPARATIVE STUDIES ON THE DIFFERENTIAL EXPRESSION OF THE C-SRC GENE (MEETING ABSTRACT)

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XXII:h Symposium for Comparative Research on Leukemia and Related Diseases, July 7-11, Hamburg, p. 67, 1985.

By immunoprecipitation and hybridization experiments we could show that the cellular homologue of the oncogene v-src of Rous sarcoma virus is probably present in all multicellular animals. Quantitative determination of the activity of the c-src encoded protein kinase during embryogenesis of fish, frog and chicken indicated a low expression of c-src during early development (cleavage stages to late neurula stages), which increases during organogenesis and decreases thereafter to the level found in adult animals. In the frog an

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additional peak in kinase activity appears during metamorphosis. In contrast to the results found in the vertebrate system, pp60c-src kinase activity is barely detectable during embryonic development of the sea urchin. Our data suggest that the c-src gene product plays more a role in differentiation than in proliferation processes. Preliminary studies on the expression of c-src during differentiation of the monomyelocytic cell line HL-60 into macrophages support this theory as TPA-treated cells show a differentiation-dependent increase of pp60c-src kinase activity.

38. DIFFERENTIAL EXPRESSION OF THE CELLULAR SRC GENE DURING VERTEBRATE DEVELOPMENT

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Dev Biol; 105(2):415-22 1984

Cellular genes that are homologous to the transforming genes of certain RNA tumor viruses are suspected to play a functional role during normal developmental processes. To investigate this further, we are studying the expression of the cellular homolog of the Rous sarcoma virus transforming gene (c-src) during embryogenesis of fish, frog, and chicken by quantitative determination of the activity of the c-src encoded protein kinase (pp60c-src). The kinase activity from embryos of fish, frog, and chicken displays the same enzymatic characteristics as the kinase from adult animals: It phosphorylates only tyrosine residues in protein substrates, and its activity is relatively insensitive to inhibition by the diadenosine nucleotide Ap4A. During the course of development, the varying kinase activity level reflects differential expression of the c-src gene product. The kinase activity is low during early development, increases dramatically during organogenesis, and decreases thereafter to the level found in adult animals. The kinase activity displays an organ specificity, with brain showing the highest activity in embryos as well as in adults. Muscle, however, shows high activities during organogenesis, but no or barely detectable activity in adult animals. Our data suggest, therefore, that the c-src gene product plays more of a role in differentiation than in proliferation processes during embryogenesis, and that it may act as a pleiotropic effector.

39. THE DIFFERENTIAL EXPRESSION OF THE CELLULAR SRC-GENE PRODUCT PP60SRC AND ITS PHOSPHOKINASE ACTIVITY IN NORMAL CHICKEN CELLS AND TISSUES

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Biochim Biophys Acta; 782(1):94-102 1984

Chick embryos of different ages and adult chickens were examined for the expression of pp60c-src, the normal cellular homolog of the transforming protein of Rous sarcoma virus. In any brain extract, the pp60c-src kinase activity was always high, whereas muscle extracts of embryos show an age-dependent decrease in kinase activity. Adult animals show either no or barely measurable activity in muscle tissue. In contrast, liver cell extracts of embryos show an age-dependent increase in pp60c-src kinase activity, with adult chickens displaying the highest activity, very similar to that found in brain extracts. This demonstrates that increased expression of c-src is not necessarily correlated with cell proliferation, but suggests that, at an early stage of differentiation of mesenchymal cells, the relatively high expression of c-src could be responsible, at least in part, for the control of cell metabolism and proliferation.

40. EXPRESSION OF PP60C-SRC PROTEIN KINASE IN ADULT AND FETAL HUMAN TISSUE: HIGH ACTIVITIES IN SOME SARCOMAS AND MAMMARY CARCINOMAS

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Cancer Res; 43(4):1696-1702 1983

An endogenous protein of human cells pp60c-src, which is closely related to the product of the transforming gene of Rous sarcoma virus, pp60v-src, has been quantitated by measuring its enzymatic activity in an immunoglobulin G protein kinase assay. The influence of normal developmental processes on pp60c-src expression was assessed by comparative analysis of various adult and fetal human tissues. The maximal difference detected was a two- to three-fold-enhanced activity in fetal muscle compared with adult muscle. Organ-specific variations in the enzyme level were observed. Highest activity was found in brain, followed by kidney, lung, muscle, and connective tissue. Since overexpression of the cellular counterparts of viral-transforming genes may play a role in carcinogenesis, pp60c-src kinase was measured in nine spontaneous human sarcomas and 21 mammary carcinomas. Compared with the respective normal tissues and human diploid fibroblasts, 4- to 20-fold-enhanced activities were observed in one-third of the sarcomas and carcinomas. The remainder showed no or insignificantly elevated activity. The enzymes from normal and malignant tissues were indistinguishable from the virus-coded enzyme with respect to specificity for divalent cations and a predominance of phosphorylation in tyrosine. Patients carrying tumors with high pp60c-src protein kinase activity did not develop kinase-reactive antibodies against pp60c-src or pp60v-src. (Author abstract) (19 Refs)

41. PP60C-SRC IN THE DEVELOPING CEREBELLUM

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Mol Cell Biol; 5(1):27-32 1985

pp60c-src was localized in the cerebellum of developing chicken embryos by immunoperoxidase staining with antisera raised against bacterially expressed pp60v-src. Immunoreactivity (IR) appeared in the cerebellum of the chicken embryos at the time of neuronal differentiation. pp60c-src IR was detected in regions of the developing cerebellum where processes of developing neurons and glia are located. In the early embryo (stage 17), pp60c-src IR was localized in the marginal zone of the cerebellar plate. By stage 40, pp60c-src IR was localized in the process-rich molecular layer of the cerebellum and between the cells of the developing internal granular layer. Cell bodies of cerebellar neurons did not show pp60c-src IR at any stage of development. Mitotically active neuroepithelial cells of the metencephalon did not express pp60c-src before the onset of differentiation in the early embryo, nor did proliferating cells of the external granular layer express pp60c-src at later stages. Although it is not possible to ascertain whether pp60c-src is localized in developing neurons or glia at the light microscope level, the time of its appearance and pattern of distribution in the molecular layer is suggestive of a localization within the developing neuronal processes which compose the bulk of this layer. Biochemical analyses of pp60c-src in the developing cerebellum by the immune complex protein kinase activity and sensitivity of the kinase to inhibition by P1,P4-di(adenosine-5')tetraphosphate confirmed that the expression of pp60c-src coincided with the time of neuronal differentiation.(ABSTRACT TRUNCATED AT 250 WORDS)

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42. OVEREXPRESSION OF THE C-SRC PROTEIN DOES NOT INDUCE TRANSFORMATION OF NIH 3T3 CELLS

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Proc Natl Acad Sci USA. 81(22):7071-5 1984

NIH 3T3 mouse cells were transfected with plasmids that induce efficient expression of either (i) the Rous sarcoma virus v-src gene, (ii) the chicken c-src gene, or (iii) a recombinant gene combining the 5' portion of c-src with the 3' end of v-src. Focus formation in tissue culture and formation of large colonies in soft agar did not occur in cells transfected with c-src. Cells transfected with c-src expression plasmids did not form foci but were isolated using a coselectable biological marker. They display morphological and substrate-independent growth characteristics intermediate between those of normal and v-src-transformed mouse cells, and lysates from these cells have enhanced in vitro tyrosine kinase activity. Transfection with the c-src-v-src recombinant induced focus formation with an efficiency similar to that obtained with a v-src expression plasmid. These results imply that v-src-induced transformation does not result just from overexpression of an essentially normal cellular protein but, at least in part, depends on the mutations distinguishing the cellular and viral proteins.

43. OVEREXPRESSED PP60C-SRC CAN INDUCE FOCUS FORMATION WITHOUT COMPLETE TRANSFORMATION OF NIH 3T3 CELLS

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Mol Cell Biol. 5(5):1073-83 1985

NIH 3T3 cells were transfected with plasmids containing Moloney murine leukemia virus long terminal repeats and either chicken c-src or v-src genes. In contrast with the effects observed after transfection with plasmids containing c-src and avian retrovirus or simian virus 40 promoter-enhancers (H. Hanafusa, H. Iba, T. Takeya, and F. R. Cross, p. 1-8, in G. F. Vande Woude, A. J. Levine, W. C. Topp, and J. D. Watson, ed., Cancer Cells, vol. 2, 1984; H. Iba, T. Takeya, F. R. Cross, T. Hanafusa, and H. Hanafusa, Proc. Natl. Acad. Sci. U.S.A. 81:4424-4428, 1984; R. C. Parker, R. Swanstrom, H. E. Varmus, and J. M. Bishop, p. 19-26, in G. F. Vande Woude et al., ed., Cancer Cells, vol. 2, 1984; R. C. Parker, H. E. Varmus, and J. M. Bishop, Cell 37:131-139, 1984; D. Shalloway, P. M. Coussens, and P. Yaciuk, p. 9-17, in G. F. Vande Woude et al., ed., Cancer Cells, vol. 2, 1984; D. Shalloway, P. M. Coussens, and P. Yaciuk, Proc. Natl. Acad. Sci. U.S.A. 81:7071-7075; and K. C. Wilhelmsen, W. G. Tarpley, and H. M. Temin, p. 303-308, in G. F. Vande Woude et al., ed., Cancer Cells, vol. 2, 1984), we found that both types of Moloney murine leukemia virus long terminal repeat-src expression plasmids induced focus formation, although c-src induced only 1% as many foci as v-src. The focus-selected c-src overexpressed cells had altered morphology and limited growth in soft agarose but were not tumorigenic in vivo. Cleveland digests, comparative in vitro kinase assays, secondary transfections, and immunoprecipitations indicated that focus formation was caused by rare transfection events that resulted in very high-level pp60c-src expression rather than by mutations of the transfected c-src genes. These results suggest that pp60v-src induced transformation is not a completely spurious activity which is unrelated to the function of pp60c-src but that it represents a perturbation of already existent molecular control processes involving pp60c-src.

44. POLYOMA VIRUS TRANSFORMING PROTEIN ASSOCIATES WITH THE PRODUCT OF THE C-SRC CELLULAR GENE

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Nature. 303(5916):435-9 1983

Polyoma virus can transform the growth properties of rodent cells grown in culture and form tumours in susceptible animals, an activity largely due to one of the virus-encoded proteins, called middle T. Middle T has an associated tyrosine-specific protein kinase activity in vitro and interacts with cellular membranes, but the biochemical basis of its ability to transform remains unclear. Although there is some correlation between the transforming activity of different polyoma virus mutants and their ability to accept phosphate on tyrosine in middle T in the in vitro kinase reaction, the abundance of phosphotyrosine in protein is not elevated in polyoma virus-transformed cells and no cellular substrates for the putative kinase have been identified. It is also not yet known whether the tyrosine kinase of middle T is an intrinsic activity of the protein itself or the property of an associated enzyme. The experiments described here indicate that a fraction of middle T forms a stable complex with pp60c-src, the product of a cellular oncogene, and lead us to propose that the middle T associated kinase at least in part is a property of pp60c-src rather than middle T itself.

45. IDENTIFICATION OF A FISH PROTEIN ASSOCIATED WITH A KINASE ACTIVITY AND RELATED TO THE ROUS SARCOMA VIRUS TRANSFORMING PROTEIN

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Cancer Res. 42(6):2429-2433 1982

Cells of all vertebrates thus far investigated contain a cellular src gene, which appears to be a highly conserved homolog of the Rous sarcoma virus oncogene src. Similar to the Rous sarcoma virus oncogene src, this endogenous cellular src gene codes for a Mr 60,000 phosphoprotein (pp60c-src) which is associated with a kinase activity. This kinase specifically phosphorylates tyrosine residues of proteins. We have systematically looked for pp60c-src in specific strains of certain teleostean fish, including the genera Xiphophorus and Girardinus. These strains are known to contain chromosomal oncogenes causing neoplastic transformation in certain mutants and hybrids. Since the pp60c-src found in other species has been shown to be antigenically related to the viral src gene product, a phosphoprotein with a molecular weight of 60,000 (pp60r-src), we have used tumor-bearing rabbit serum which recognizes Rous sarcoma virus pp60src to detect such a related protein in fish. Tumor-bearing rabbit serum precipitated two proteins with molecular weights of 60,000 and 52,000. The Mr 60,000 phosphoprotein was associated with a protein kinase activity that phosphorylates tyrosine residues of the immunoprecipitating immunoglobulin G heavy chain (Mr 53,000). The antigenic relationship of this fish protein to viral pp60src was further documented by an immune competition assay. We conclude, therefore, that the fish Mr 60,000 phosphoprotein is a pp60c-src encoded for by a fish cellular src gene that corresponds to the viral src oncogene. The Mr 52,000 phosphoprotein is probably a proteolytic degradation product of pp60c-src. All animals of the various strains of the fish investigated in this study showed kinase activity that was reactive upon immunoprecipitation with tumor-bearing rabbit serum, indicating that the pp60c-src protein is a normal constituent of the fish. Measurable quantitative differ-

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ences were revealed between the various strains as well as in different tissues. Whereas brain and melanoma tissue showed a high kinase activity when compared to skin, liver, spleen, and testes, muscle tissue displayed no significant activity. (Author abstract) (27 Refs)

46. CORRELATIONS OF INHERITANCE AND EXPRESSION BETWEEN A TUMOR GENE AND THE CELLULAR HOMOLOG OF THE ROUS SARCOMA VIRUS-TRANSFORMING GENE IN XIPHOPHORUS

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Cancer Res; 42(10):4222-4227 1982

Neoplastic transformation of pigment cells in the teleostean fish Xiphophorus is mediated by a cellular oncogene (Tu). Normally, Tu is suppressed by multiple regulating genes (R). Depending on impairment and loss of R genes, Tu is permitted to express itself phenotypically. In the pigment cell system, different degrees of Tu expression lead to small spots of transformed cells or to benign or malignant melanoma. All neoplastic and nonneoplastic cells of all Xiphophorus genotypes tested thus far appear to contain the cellular homolog (c-src) of the avian sarcoma virus oncogene (v-src). The evidence for this stems from the detectability of a Mr 60,000 phosphoprotein with associated kinase activity (pp60c-src) that reacts with antiserum against viral pp60src. We followed the inheritance of Tu (identified by spots and melanomas) compared to the expression of c-src (identified by the pp60c-src-associated protein kinase). By quantitative determination of kinase activity in immunoprecipitated pp60c-src from fish showing different degrees of Tu expression, we have investigated whether there exists a correlation between the expression of c-src and Tu. In genotypes with the same genetic background, cells from Tu-containing fish express more pp60c-src than do cells from fish lacking Tu. In genotypes carrying a Tu gene and which show differences in the amount of gene expression due to a different extent of repression by regulating genes, analysis of kinase activity revealed that an increase of Tu expression is correlated with an elevated level of pp60c-src-associated kinase activity. Our findings may indicate that c-src activity in Xiphophorus is modulated by the Tu gene product or that Tu and c-src are regulated coordinately. (Author abstract) (26 Refs)

47. ELEVATED EXPRESSION OF THE CELLULAR SRC GENE IN TUMORS OF DIFFERING ETIOLOGIES IN XIPHOPHORUS

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Int J Cancer; 36(2):199-207 1985

In the fish Xiphophorus we have detected elevated levels of pp60c-src kinase activity in a variety of tumors (n = 34) of neurogenic, epithelial, and mesenchymal origin either of hereditary etiology or induced by carcinogens. This elevation ranged from 2-fold up to 50-fold compared to the corresponding non-tumorous tissue and up to 6-fold compared to the highest activities found in any of the normal organs. The level of elevation parallels the degree of malignancy in melanoma and in tumors of mesenchymal origin. In fish bearing tumors of hereditary etiology kinase activity was also elevated in the non-tumorous brain, while in fish bearing induced tumors, kinase activity was elevated only in the cells of the neoplasia.

48. THE PRODUCT OF THE PROTOONCOGENE C-SRC IS MODIFIED DURING THE CELLULAR RESPONSE TO PLATELET-DERIVED GROWTH FACTOR

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Proc Natl Acad Sci USA; 82(23):7845-9 1985

We have observed a modification of the cellular protein kinase pp60c-src, elicited in murine 3T3 fibroblasts by platelet-derived growth factor (PDGF). The modification occurred rapidly after addition of PDGF to the culture medium and was first detected as a reduction in the electrophoretic mobility of a portion of the pp60c-src molecules. A similarly modified form of the viral homologue pp60v-src occurs in vivo in the absence of stimulation by PDGF. The occurrence of modified forms of both pp60c-src and pp60v-src was associated with a novel phosphorylation at tyrosine in the amino-terminal domains of the proteins. The time-course and dose-response for this modification of pp60c-src paralleled PDGF-induced increases in phosphorylation of pp36, a major cellular substrate for several tyrosine-specific protein kinases. In parallel experiments, treatment of cells with PDGF increased the kinase activity of pp60c-src in an immunocomplex assay. These results suggest pp60c-src may play a role in the mitogenic response to PDGF.

49. SELF-RENEWAL OF HAEMOPOIETIC STEM CELLS: THE ROLES OF THE ENVIRONMENT, OF GROWTH FACTORS AND OF THE SRC ONCOGENE

Dexter TM. Boettiger D. Spooncer E

Department of Experimental Haematology, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Withington, Manchester, England.

Hematol Bluttransfus; 29:363-71 1985

Keywords (MeSH): Animal; Bone Marrow/cytology; Cell Division; Cell Line; Cell Transformation, Neoplastic; Growth Substances/*physiology; Hematopoietic Stem Cells/*cytology; Human; Leukemia/familial and genetic/*pathology; *Oncogenes; Tissue Culture/methods.

50. INTERACTION OF THE ROUS SARCOMA VIRUS PROTEIN PP60SRC WITH THE CELLULAR PROTEINS PP50 AND PP90

Brugge JS

Curr Top Microbiol Immunol; 123:1-22 1986

Keywords (MeSH): Animal; Avian Sarcoma Viruses/*enzymology; Binding Sites; Biological Transport; Cell Membrane/metabolism; *Cell Transformation, Viral; Macromolecular Systems; Molecular Weight; Mutation; Myristates/metabolism; Oncogene Proteins, Viral/*metabolism; Phosphoproteins/*metabolism; Phosphorylation; Phosphotransferases/metabolism; Protein-Tyrosine Kinase/*metabolism; Retroviridae Proteins/genetics/*metabolism; Tyrosine/analogs and derivatives/metabolism.

51. TOWARDS A BIOCHEMICAL DESCRIPTION OF MALIGNANT TRANSFORMATION. IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF THE ROUS SARCOMA VIRUS TRANSFORMING GENE PRODUCT (14 Refs)

Erikson RL

Cancer; 53(10):2041-5 1984

Keywords (MeSH): Animal; Avian Sarcoma Viruses/*genetics; Cats; *Cell Transformation, Neoplastic; *Cell Transformation, Viral; Feline Fibrosarcoma Virus/genetics; Gene Expression Regulation; *Genes, Viral; Mice; Mouse Leukemia Viruses/genetics; Mouse Sarcoma Viruses/genetics; *Oncogenes; Rabbits; Review; Viral Proteins/genetics.

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2.

erb B protein product

52. SIMILARITY OF PROTEIN ENCODED BY THE HUMAN C-ERB-B-2 GENE TO EPIDERMAL GROWTH FACTOR RECEPTOR

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Nature; 319(6050):230-4 1986

A novel v-erb-B-related gene, c-erb-B-2, which has been identified in the human genome, maps to human chromosome 17 at q21 (ref. 40), and seems to encode a polypeptide with a kinase domain that is highly homologous with, but distinct from, that of the epidermal growth factor (EGF) receptor. The c-erb-B-2 gene is conserved in vertebrates and it has been suggested that the neu gene, detected in a series of rat neuro/glioblastomas, is, in fact, the rat c-erb-B-2 gene. Amplification of the c-erb-B-2 gene in a salivary adenocarcinoma and a gastric cancer cell line MKN-7 suggests that its over-expression is sometimes involved in the neoplastic process. To determine the nature of the c-erb-B-2 protein, we have now molecularly cloned complementary DNA for c-erb-B-2 messenger RNA prepared from MKN- 7 cells. Its sequence shows that the c-erb-B-2 gene encodes a possible receptor protein and allows an analysis of the similarity of the protein to the EGF receptor and the neu product. As a consequence of chromosomal aberration in MKN-7 cells, a 4.6-kilobase (kb) normal transcript and a truncated 2.3-kb transcript of c-erb-B-2 are synthesized at elevated levels. The latter transcript presumably encodes only the extracellular domain of the putative receptor.

53. CHARACTERIZATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR AND THE ERBB ONCOGENE PRODUCT BY SITE-SPECIFIC ANTIBODIES

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Arch Biochem Biophys; 249(2):531-6 1986

Site-specific antibodies to the src-homologous domain (residues 373-383) of the erbB gene product neutralized the tyrosine kinase activity of the epidermal growth factor receptor, suggesting that the region against which the antibodies were directed may be functionally important for the kinase activity. In the immunofluorescence experiment, the site-specific antibodies detected the epidermal growth factor receptor and the erbB gene product only when the cells were permeabilized prior to staining, while monoclonal anti-epidermal growth factor receptor antibody, which recognizes the epidermal growth factor binding domain, gave a positive surface stain with viable nonpermeabilized A431 cells. This result supports the view that the epidermal growth factor binding domain and the src-homologous domain are located at the cell surface and inner face of the plasma membrane, respectively.

54. THE ONCOGENE PRODUCTS ERBB AND MIL/RAF (MEETING ABSTRACT)

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XXIIth Symposium for Comparative Research on Leukemia and Related Diseases, July 7-11, Hamburg, p. 51, 1985.

Avian erythroblastosis virus codes for the oncogenes gag-erbA and erbB. Using antibodies against gag and the bacterially expressed erbB as well as against erbB-specific synthetic peptides, we purified the two gene products about 3,000 fold and characterized them. While no enzymatic function could be attributed to gag-erbA, the erbB autophosphorylates its two predominant forms gp68 and gp74. Phosphorylation occurred in the amino acid tyrosine. Tyrosine-phosphorylation of the EGF-receptor was detectable with the anti-erbB sera as well. The oncogenes of the MH2 virus, gag-mil and myc, were also characterized. The gag-mil protein exhibits a serine/threonine-specific protein kinase activity in autophosphorylation reactions as well as on exogenous substrates such as histone H5 and actin. The highly related raf also exhibits a serine/threonine-specific protein kinase activity (Moelling et al, Nature 312, 558, 1984). Ts mutant studies of MH2 are under way. Lipid phosphorylation does not appear to be an intrinsic property of tyrosine or serine/threonine protein kinases. Another model for the potential role of tyrosine and serine/threonine kinases in malignant transformation was proposed. (1 Ref)

55. [ROLE OF INOSITOL PHOSPHOLIPIDS METABOLISM IN THE SIGNAL TRANSDUCTION OF ERBB GENE PRODUCT]

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Gan To Kagaku Ryoho; 13(3 Pt 2):854-60 1986

We have observed the enhanced metabolism of inositol phospholipids in erbB transformed chicken fibroblasts. This increased metabolism seemed to be due to the activation of PI-kinase, DPI-kinase and DG-kinase activities in these cells. And also, C-kinase activity in the transformed cells was observed to be sustained as an active form. These results suggest the signal transduction system through the enhanced metabolism of inositol phospholipids was activated in the transformed cells. When the normal cells were treated with the combination of TPA, which is an activator of C-kinase, and Ca2+-ionophore A23187, the stimulation of DNA synthesis was detected. However, such treatment caused the only inhibitory effect on the cell growth of the transformed cells. On the other hand, the erbB gene product by itself dose not have significant activity of PI-kinase, DPI-kinase and DG-kinase. Therefore, erbB gene product may indirectly enhance inositol-phospholipids metabolism and induce abnormal growth of transformed cells.

56. ANALYSIS OF A TYROSINE-SPECIFIC PROTEIN KINASE ACTIVITY ASSOCIATED WITH THE RETROVIRAL ERBB ONCOGENE PRODUCT

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Exp Cell Res; 161(1):199-208 1985

The transforming protein erbB of avian erythroblastosis virus (AEV) has considerable sequence homology with the epidermal growth factor (EGF) and appears to represent a truncated form of this receptor. The sequence of the erbB gene is furthermore related to that of other viral transforming genes such as src, fps, yes or abl. The transforming proteins of these src-related oncogenes as well as receptors for EGF, platelet-derived growth factor (PDGF), and insulin are associated with tyrosine-specific protein kinases. It has been difficult to demonstrate this activity for the erbB protein. To analyze the erbB gene product, we prepared polyclonal antibodies against a bacterially expressed erbB DNA restriction fragment (BamHI/BamHI). The antiserum is shown to immunoprecipitate the erbB protein from AEV-transformed chicken fibroblasts and also recognizes the EGF receptor protein. Both proteins become phosphorylated in vitro on tyrosine residues upon the addition of [gamma-32P]ATP. The

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protein kinase activity is low compared to other oncogene-specific kinases. This is not due to kinase block ing by the serum, because erbB carboxyterminal synthetic peptide antibodies give rise to low levels of protein kinase activity as well indicating that this may be a characteristic property of erbB in vitro.

57. A V-ERBB-RELATED PROTOONCOGENE, C-ERBB-2, IS DISTINCT FROM THE C-ERBB-1/EPIDERMAL GROWTH FACTOR-RECEPTOR GENE AND IS AMPLIFIED IN A HUMAN SALIVARY GLAND ADENOCARCINOMA

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Proc Natl Acad Sci USA; 82(19):6497-501 1985

From a human genomic library, we obtained six v-erbB-related DNA clones. A DNA probe prepared from one of the clones, lambda 107, hybridized to EcoRI fragments of 6.4 and 13 kilobase pairs of human DNA. Neither of these fragments was amplified in A431 vulva carcinoma cells, in which the gene encoding the epidermal growth factor receptor is amplified. In addition, the probe from lambda 107 hybridized with a single, 4.8-kilobase poly(A)+ RNA species and did not react with EGF receptor mRNA. Thus, we conclude that clone lambda 107 represents a v-erbB-related gene (c-erbB-2) that is distinct from the EGF receptor gene. In contrast, the other five clones were shown to represent the EGF receptor gene (c-erbB-1). Partial nucleotide sequence analysis of the lambda 107 insert showed that this clone contained at least seven putative exons and that six of them could encode the kinase domain characteristic of protein products of the src oncogene family. Southern blot analysis showed close similarity of the restriction patterns of the rat c-erbB-2 gene and the rat neu oncogene, suggesting possible involvement of c-erbB-2 in human cancer. In fact, approximately 30-fold amplification of c-erbB-2 was observed in a human adenocarcinoma of the salivary gland.

58. IDENTIFICATION OF A FORM OF THE AVIAN ERYTHROBLASTOSIS VIRUS ERB-B GENE PRODUCT AT THE CELL SURFACE

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Nature; 309(5967):460-2 1984

Avian erythroblastosis virus (AEV) induces both erythroblastosis and fibrosarcoma in chickens. The viral oncogene responsible for these diseases, erb, is divided into two regions, erb-A and erb-B, although recent evidence suggests that it is primarily the erb-B gene product that is responsible for the transforming activity. The erb-B gene product has been reported previously to be a membrane glycoprotein of 68,000 molecular weight (MW), gp68erb-B. However, we show here that gp68erb-B is an intracellular precursor which is modified further to a 74,000 MW protein, gp74erb -B. By the criteria of resistance to digestion with endoglycosidase H, subcellular fractionation and inhibition of biosynthesis by the ionophore monensin, gp74erb -B appears to be located at the cell surface. Recently, a comparison of the erb-B sequence with that of the epidermal growth factor (EGF) receptor has shown tha t these two genes are highly homologous, and that erb-B appears to represent a truncated form of this growth factor. In light of these data the identification of gp74erb -B at the plasma membrane suggests that this may be the functionally important form of the erb-B gene product.

59. THE MEMBRANE GLYCOPROTEIN ENCODED BY THE RETROVIRAL ONCOGENE V-ERB-B IS STRUCTURALLY RELATED TO TYROSINE-SPECIFIC PROTEIN KINASES

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Proc Natl Acad Sci USA; 81(3):704-7 1984

Avian erythroblastosis virus can transform both fibroblasts and erythroid cells to neoplastic growth. A locus within the virus genome, v-erb B, encodes a membrane glycoprotein essential for the oncogenic properties of the virus. No biochemical function has until now been attributed to the v-erb B protein. We report here that the v-erb B glycoprotein shares strong structural homologies with the tyrosine-specific protein kinases encoded by certain retroviral oncogenes. The patterns of amino acid conservation between the tyrosine-specific protein kinases and the v-erb B protein, and between the v-erb B protein and the catalytic subunit of bovine protein kinase, suggest a possible functional as well as structural relatedness.

60. ANTIBODIES AGAINST A SYNTHETIC PEPTIDE AS A PROBE FOR THE KINASE ACTIVITY OF THE AVIAN EGF RECEPTOR AND V-ERBB PROTEIN

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Cell; 40(3):619-25 1985

The transforming protein v-erbB of avian erythroblastosis virus (AEV) displays extensive sequence homology with the presumptive protein-tyrosine kinase domain of the human EGF receptor and with the src protein-tyrosine kinase family of oncogenes. However, no kinase activity has previously been demonstrated for the v-erbB protein. Here antibodies generated against a synthetic peptide from the C terminus of human EGF receptor are shown to immunoprecipitate the EGF receptor from human and avian cells, as well as the v-erbB proteins from AEV-transformed cells that become phosphorylated on tyrosine residues upon the addition of gamma-32P-ATP. The immunoprecipitates are also able to phosphorylate exogenous tyrosine-containing substrates. Hence, it is likely that both avian EGF receptor and v-erbB proteins are protein tyrosine-specific protein kinases. Since the kinase activity of v-erbB protein cannot be regulated by EGF, it is proposed that the tyrosine protein kinase function of v-erbB may be constitutively activated.

61. ROUS-ASSOCIATED VIRUS 1-INDUCED ERYTHROLEUKEMIC CELLS EXHIBIT A WEAKLY TRANSFORMED PHENOTYPE IN VITRO AND RELEASE C-ERBB-CONTAINING RETROVIRUSES UNABLE TO TRANSFORM FIBROBLASTS

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J Virol; 57(3):1127-38 1986

Avian leukosis viruses induce erythroblastosis in chicks by integrating into the c-erbB gene and thus activating c-erbB transcription. We characterized Rous-associated virus 1-induced leukemic erythroblasts in vitro and showed that they mostly resemble erythropoietin-independent but otherwise normal erythroid progenitors. Some leukemic cells, however, were able to both differentiate and proliferate extensively in vitro. All 14 leukemias studied expressed high levels of erbB-related proteins that were 5 to 10 kilodaltons larger but otherwise very similar to the gp74erbB protein of avian erythroblastosis virus ES4 with respect to biosynthesis, glycosyla-

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tion, and cell surface expression. Two leukemias contained and released retroviruses that transduced erbB. Chicken embryo fibroblasts fully infected with these viruses expressed high levels of erbB RNA and protein but retained a normal phenotype. Our results suggest that certain forms of c-erbB, activated by long terminal repeat insertion or viral transduction, are capable of inducing erythroleukemia but unable to transform fibroblasts.

62. CHARACTERIZATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR

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Growth and Differentiation of Cells in Defined Environment
Murakami H, Yamane I, Barnes DW, Mather JP, Hayashi I, Sato GH, eds. New York, Springer-Verlag, p. 405-10, 1985

A strong link between hormonal control of cell growth and tumorigenesis is evident from findings that the v-sis oncogene is platelet-derived growth factor and that there is strong protein sequence homology between the v-erb B oncogene and the epidermal growth factor (EGF) receptor. A monoclonal anti-EGF receptor antibody was used to purify enzymatically active EGF receptor for study of its kinetic properties, and a polyclonal antibody generated against purified EGF receptor protein was used to clone, by expression cloning methodology, the EGF receptor for study of its structure. A series of monoclonal antibodies was prepared against EGF receptors from human epidermoid carcinoma A431 cells, a rich source of human EGF receptor protein. Three of these antibodies were competitive inhibitors of 125I-EGF binding to the A431 cells, indicating that the antibodies were directed against the EGF binding site of the receptor protein. The competitive antagonist character of these three antibodies was confirmed by showing that they reversibly inhibited EGF-stimulated growth of human fibroblasts. The EGF receptor is a phosphoprotein. In response to EGF in vivo, self-phosphorylation occurs predominantly at a single tyrosine residue. The EGF receptor also contains phosphoserine and phosphothreonine. Phosphorylation at threonine residues occurs in response to tumor promoters (and EGF) in vivo and to protein kinase C in vitro; this results in inhibition of EGF-dependent tyrosine kinase activity, an apparent autoregulatory feedback mechanism. Using clonal cDNA probes from different regions, the RNA products of EGF receptor gene expression were analyzed; two cytoplasmic poly(A)+ RNAs of 10.6 and 6.3 kilobase were detected, using clonal probes containing sequences in the tyrosine kinase domain. Functional organization of the EGF receptor protein is described. (22 Refs)

63. ONCOGENE EXPRESSION AND THE ROLE OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN THE TERMINAL DIFFERENTIATION OF CULTURED HUMAN MALIGNANT KERATINOCYTES (SQCC/Y1) (MEETING ABSTRACT)

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Proc Annu Meet Am Assoc Cancer Res, 26:40 1985

The SqCC/Y1 human keratinocyte line, derived from a carcinoma of the stratified squamous epithelium of the cheek, has been shown by our laboratory to express several phenotypic properties of normal epidermis upon terminal differentiation. Serum deprivation of hydrocortisone treatment induce differentiation of SqCC/Y1, as measured by the formation of cornified cell envelopes, and the keratinization of the cells. SqCC/Y1 cells express the oncogenes c-erb B, c-myc, c-abl, and c-sis. The expression of c-erb B in cells cultured in serum-free medium for either 3 or 6 days was found using an RNA dot-blot assay to be reduced to 33% and 25%, respectively, of the undifferentiated level. Similarly, c-myc expression was also decreased as a result of maturation. No change occurred in the copy number of myc and erb B at the DNA level, as measured by Southern blotting. C-erb B, the cellular homolog of the transforming gene of the avian erythroblastosis virus, has been demonstrated to code for part of EGFR. Measurement of the phosphorylation of EGFR in vitro was performed on isolated cell membranes prepared from both differentiated and undifferentiated cultures. The degree of phosphorylation was considerably reduced in differentiated cultures, suggesting that either the amount of EGFR or the cell surface was decreased or EGFR may not be completely accessible for phosphorylation in mature cultures. Consistent with these findings supporting the importance of EGFR to the processes of proliferation and maturation, EGF at physiological concentrations inhibited terminal differentiation and stimulated the cellular proliferation of SqCC/Y1 cells in serum-free medium. The results suggest that the expression of the c-erb B and c-myc oncogenes and of EGF and its receptor are important for the regulation of SqCC/Y1 cell proliferation and differentiation.

64. EXPRESSION OF CELLULAR ONCOGENES IN HUMAN EMBRYONIC AND FETAL HEMOPOIETIC CELLS (MEETING ABSTRACT)

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XV Meeting of the European Tumour Virus Group, September 19-24, 1984, Urbino, Italy, p. 10, 1984

A role for human cellular oncogenes (c-onc) in the control of normal cell proliferation and differentiation, although generally postulated, is still not proven. In this regard, growing evidence indicates that c-onc may be related to growth factors: thus, c-sis codes for the platelet-derived growth factor and v-erb B for a product partially homologous with the receptor for the epidermal growth factor. In the avian and murine model, embryonic cells express elevated levels of various c-onc, as compared to corresponding normal adult cells: this suggests a direct link between c-onc expression and cell proliferation and differentiation in ontogeny. We have established a large collection of human embryonic and fetal hemopoietic and non-hemopoietic cells, obtained from legal curettage abortions in the 5-12 wk post-conception period. The expression of c-myc, c-myb, c-abl, c-erb A and B, c-fes, c-sis, c-mos, c-ras-H and c-ras-K has been evaluated in purified yolk sac and liver hemopoietic (mainly erythroblastic) cells by cytoplasmic dot and Northern blot analysis, followed by hybridization with human specific genomic or cDNA probes. Our data indicate that c-erb B is expressed at elevated levels in both embryonic and fetal erythroblasts as compared with normal adult erythroblasts. In K562 human erythroleukemic line this gene is overexpressed, although neither amplified nor grossly rearranged, as evaluated by Southern blot analysis. These data may suggest a role for c-erb B in the control of the kinetics of human erythropoietic cells, particularly in conditions associated with enhanced or abnormal erythroid proliferation. Further data will be presented on embryonic and fetal erythroblasts fractionated on density gradients, as well as on various embryonic and fetal non-hemopoietic cells and tissues, with respect to both c-erb B and other oncogenes.

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65. SEPARATION AND CHARACTERIZATION OF A PHOSPHATIDYLINOSITOL KINASE ACTIVITY THAT CO-PURIFIES WITH THE EPIDERMAL GROWTH FACTOR RECEPTOR

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J Biol Chem: 260(15):8824-30 1985

Two retroviral protein-tyrosine kinases, v-src and v-ros, have been reported to possess phosphatidylinositol (PtdIns) kinase activity. Because the epidermal growth factor (EGF) receptor is a protein-tyrosine kinase with structural homology to p60v-src and because EGF stimulates PtdIns turnover in A431 cells, the EGF receptor has been examined for PtdIns kinase activity. Preparations of the EGF receptor, isolated from A431 cells and purified by two different methods of affinity chromatography, possessed an associated PtdIns kinase activity. This activity which co-purified with the EGF receptor represented only about 2% of the total PtdIns kinase activity of A431 membranes, and there was no correlation between the number of EGF receptors and the amount of PtdIns kinase activity in membranes from various cell types. A peptide substrate, angiotensin II, and PtdIns did not compete with each other as substrates for the protein-tyrosine and PtdIns kinase activities of the EGF receptor. When self-phosphorylated EGF receptor was fractionated by Sephacryl S-300 gel permeation chromatography, the peak of PtdIns kinase activity was separated from the comigrating peak of protein-tyrosine kinase activity and the self-phosphorylated EGF receptor. These results indicate that the protein-tyrosine kinase and PtdIns kinase activities which co-purify with the EGF receptor reside on different molecules. Angiotensin II and PtdIns did not compete as substrates for p60v-src isolated by immunoabsorption with a monoclonal antibody, suggesting that PtdIns kinase activity may also not be intrinsic to p60v-src.

66. 42,000-MOLECULAR WEIGHT EGF RECEPTOR HAS PROTEIN KINASE ACTIVITY

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Nature: 311(5985):477-80 1984

The epidermal growth factor (EGF) receptor and other growth factor receptors have been shown to possess tyrosine-specific protein kinase activity. Before the demonstration of kinase activity in growth factor receptors, tyrosine kinases of molecular weight (MW) 60,000 (60K) were found to be encoded by the src oncogene and other oncogenes related to src. Our earlier work on intracellular processing of the EGF receptor, a 170,000-MW polypeptide, provided evidence for proteolytic separation of well defined structural domains, and suggested to us the possibility of separating functional domains by limited proteolysis. The isolation of such kinase domains should facilitate comparison of the receptor/kinase with other well characterized kinases including those of oncogene origin. We report here the identification of a catalytically functional 42K kinase derived proteolytically from the isolated human EGF receptor. This fragment, comparable in size to pp60src, carries the kinase ATP-binding site, and functions catalytically even after detachment from the EGF-binding site and the major autophosphorylation region.

67. MODELLING THE ATP-BINDING SITE OF ONCOGENE PRODUCTS, THE EPIDERMAL GROWTH FACTOR RECEPTOR AND RELATED PROTEINS

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FEBS Lett: 175(2):387-92 1984

A three-dimensional model for the ATP-binding site of the oncogene product v-src is proposed by the use of interactive computer graphics. A similar model would apply to sequence related proteins such as other oncogene products, the epidermal growth factor receptor, cAMP-dependent protein kinase and the cell division control protein CDC28. The model was proposed on the basis of the conservation of certain key residues between the oncogene product family of proteins and several nucleotide binding proteins of known structures.

68. CHARACTERIZATION OF THE NUMBER OF CARBOHYDRATE CHAINS ON THE AVIAN ERYTHROBLASTOSIS VIRUS ERB B GENE PRODUCT AND THEIR ROLE IN TRANSFORMATION

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Hamatol Bluttransfus: 29:284-9 1985

Keywords (MeSH): Animal; Avian Leukosis Viruses/*genetics; Avian Leukosis/microbiology; Carbohydrates/analysis; *Cell Transformation, Neoplastic; Chickens; Erythroblastosis Virus, Avian/*genetics; *Genes, Structural; *Genes, Viral; Molecular Weight; Viral Proteins/genetics/*isolation and purification.

3.

neu protein product

69. THE NEU GENE: AN ERBB-HOMOLOGOUS GENE DISTINCT FROM AND UNLINKED TO THE GENE ENCODING THE EGF RECEPTOR

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Science: 229(4717):976-8 1985

The neu oncogene, identified in ethylnitrosourea-induced rat neuroglioblastomas, had strong homology with the erbB gene that encodes the epidermal growth factor receptor. This homology was limited to the region of erbB encoding the tyrosine kinase domain. It was concluded that the neu gene is a distinct novel gene, as it is not coamplified with sequences encoding the EGF receptor in the genome of the A431 tumor line and it maps to human chromosome 17.

70. THE NEU ONCOGENE ENCODES AN EPIDERMAL GROWTH FACTOR RECEPTOR-RELATED PROTEIN

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Nature: 319(6050):226-30 1986

The neu oncogene is repeatedly activated in neuro- and glioblastomas derived by transplacental mutagenesis of the BDIX strain of rat with ethylnitrosourea. Foci induced by the DNAs from such tumours on NIH 3T3 cells contain the neu oncogene and an associated phosphoprotein of relative molecular mass 185,000 (p185). Previous work has shown that the neu gene is related to, but distinct from, the gene encoding the EGF receptor (c-erb-B). Here we describe a neu complementary DNA clone isolated from a cell line transformed by this oncogene; the clone has biological activity in a focus-forming assay. The nucleotide sequence of this clone predicts a 1,260-amino-acid transmembrane protein product

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similar in overall structure to the EGF receptor. We found that 50% of the predicted amino acids of neu and the EGF receptor are identical; greater than 80% of the amino acids in the tyrosine kinase dom ain are identical. Our results suggest strongly that the neu gene encodes the receptor for an as yet unidentified growth factor.

71. A V-ERBB-RELATED PROTOONCOGENE, C-ERBB-2, IS DISTINCT FROM THE C-ERBB-1/EPIDERMAL GROWTH FACTOR-RECEPTOR GENE AND IS AMPLIFIED IN A HUMAN SALIVARY GLAND ADENOCARCINOMA

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Proc Natl Acad Sci USA. 82(19):6497-501 1985

From a human genomic library, we obtained six v-erbB-related DNA clones. A DNA probe prepared from one of the clones, lambda 107, hybridized to EcoRI fragments of 6.4 and 13 kilobase pairs of human DNA. Neither of these fragments was amplified in A431 vulva carcinoma cells, in which the gene encoding the epidermal growth factor receptor is amplified. In addition, the probe from lambda 107 hybridized with a single, 4.8-kilobase poly(A)+ RNA species and did not react with EGF receptor mRNA. Thus, we conclude that clone lambda 107 represents a v-erbB-related gene (c-erbB-2) that is distinct from the EGF receptor gene. In contrast, the other five clones were shown to represent the EGF receptor gene (c-erbB-1). Partial nucleotide sequence analysis of the lambda 107 insert showed that this clone contained at least seven putative exons and that six of them could encode the kinase domain characteristic of protein products of the src oncogene family. Southern blot analysis showed close similarity of the restriction patterns of the rat c-erbB-2 gene and the rat neu oncogene, suggesting possible involvement of c-erbB-2 in human cancer. In fact, approximately 30-fold amplification of c-erbB-2 was observed in a human adenocarcinoma of the salivary gland.

72. AMPLIFICATION OF A NOVEL V-ERBB-RELATED GENE IN A HUMAN MAMMARY CARCINOMA

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Science. 229(4717):974-6 1985

The cellular gene encoding the receptor for epidermal growth factor (EGF) has considerable homology to the oncogene of avian erythroblastosis virus. In a human mammary carcinoma, a DNA sequence was identified that is related to v-erbB but amplified in a manner that appeared to distinguish it from the gene for the EGF receptor. Molecular cloning of this DNA segment and nucleotide sequence analysis revealed the presence of two putative exons in a DNA segment whose predicted amino acid sequence was closely related to, but different from, the corresponding sequence of the erbB/EGF receptor. Moreover, this DNA segment identified a 5-kilobase transcript distinct from the transcripts of the EGF receptor gene. Thus, a new member of the tyrosine kinase proto-oncogene family has been identified on the basis of its amplification in a human mammary carcinoma.

4.

ros protein product

73. SUBSTRATE SPECIFICITIES OF LIPID PHOSPHORYLATION BY TYROSINE KINASES ROS, FPS AND INSULIN RECEPTOR (MEETING ABSTRACT)

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XV Meeting of the European Tumour Virus Group. September 19-24. 1984. Urbino. Italy. p. 117. 1984.

The oncogene product of avian sarcoma virus UR2 (p68v-ros), which belongs to the 'src family' of tyrosine kinases, was recently found to be associated with a phosphatidylinositol (PI) kinase activity upon immunoprecipitation from transformed chick embryo fibroblasts with an anti-gag antibody (Macara et al, Proc Natl Acad Sci, 1984, in press). We have now explored further the phosphorylating activity associated with immunoprecipitates of p68v-ros and p130v-fps (from Fujinami sarcoma virus transformed cells) and with insulin receptor purified from rat hepatocyte membranes, using a variety of substrates. We were unable to detect any insulin-stimulated phosphorylation of PI, phosphatidylinositol phosphate (PIP) or 1,2-diacylglycerol (DAG) with insulin receptor either highly purified by affinity chromatography or as a crude lectin column eluate, under conditions in which insulin-stimulated autophosphorylation was readily apparent. Immunoprecipitates of both p68v-ros and p130v-fps showed PI kinase activity, but we were unable to detect phosphorylation of PIP, DAG or phosphatidyl glycerol. These results demonstrate that the PI kinase activities associated with immunoprecipitated p68v-ros and p130v-fps are highly substrate-specific and are therefore unlikely to be merely adventitious; and also that PI kinase activity is not a property common to all tyrosine kinases. This latter observation might prove useful in distinguishing between different functional classes of oncogene and receptor kinases. (2 Refs)

74. NUCLEOTIDE SEQUENCE OF AVIAN SARCOMA VIRUS UR2 AND COMPARISON OF ITS TRANSFORMING GENE WITH OTHER MEMBERS OF THE TYROSINE PROTEIN KINASE ONCOGENE FAMILY

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J Virol. 53(3):879-84 1985

The genome of avian sarcoma virus UR2 was completely sequenced and found to have a size of 3,165 nucleotides. The UR2-specific transforming sequence, ros, with a length of 1,273 nucleotides, is inserted between the truncated gag gene coding for p19 and the env gene coding for gp37 of the UR2AV helper virus. The deduced amino acid sequence for the UR2 transforming protein P68 gives a molecular weight of 61,113 and shows that it is closely related to the oncogene family coding for tyrosine protein kinases. P68 contains two distinctive hydrophobic regions that are absent in other tyrosine kinases, and it has unique amino acid changes and insertions within the conserved domain of the kinases. These characteristics may modulate the activity and target specificity of P68.

75. HUMAN INSULIN RECEPTOR AND ITS RELATIONSHIP TO THE TYROSINE KINASE FAMILY OF ONCOGENES

Ullrich A. Bell JR. Chen EY. Herrera R. Petruzzelli LM. Dull TJ. Gray A. Coussens L. Liao YC. Tsubokawa M. et al
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Nature. 313(6005):756-61 1985

We have deduced the entire 1,370-amino-acid sequence of the human insulin receptor precursor from a single comple-

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mentary DNA clone. The precursor starts with a 27-amino-acid signal sequence, followed by the receptor alpha-subunit, a precursor processing enzyme cleavage site, then the beta-subunit containing a single 23-amino-acid transmembrane sequence. There are sequence homologies to human epidermal growth factor receptor and the members of the src family of oncogene products.

5.

fps/fes protein product

76. TRANSFORMING POTENTIAL OF A HUMAN PROTOONCOGENE (C-FPS/FES) LOCUS

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Proc Natl Acad Sci USA; 81(10):3039-43 1984

The protooncogene c-fps/fes is a single vertebrate locus homologous to both the fps transforming gene of Fujinami avian sarcoma virus and the fes transforming gene of two feline sarcoma virus isolates. The human c-fps/fes locus has been previously cloned and characterized. We report that a recombinant gene in which 3' human c-fps/fes sequences replace over 80% of the feline sarcoma virus fes sequences transforms NIH 3T3 mouse fibroblasts and encodes a protein kinase. Cells transformed by this recombinant possess increased phosphotyrosine levels. These observations demonstrate that a large carboxyl portion of a human c-fps/fes protein product can functionally complement a retroviral transforming protein.

77. REGULATION OF EXPRESSION OF A RETROVIRAL TRANSFORMING GENE AND ITS CELLULAR HOMOLOG

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Diss Abstr Int kBl; 45(8):2405B-6B 1985

This thesis has focused on the avian Fujinami sarcoma virus (FSV) and its role in transformation. Specifically, it included the biochemical characterization of temperature sensitive (ts) mutants of FSV, the identification of the product of a cellular gene homologous to the transforming gene of FSV, and the isolation and characterization of phenotypic revertants of rat cells transformed by FSV. Analysis of the defect of the two ts mutants of FSV revealed that P130 is the transforming protein of FSV, and that its kinase activity plays a crucial role in cell transformation by this virus. v-fps, the transforming gene of FSV is homologous to a cellular gene, c-fps, present in uninfected cells. We obtained a specific antiserum directed against the v-fps domain of P130. This antiserum specifically crossreacts with a cellular protein of MW 98,000 daltons, present in uninfected chicken bone marrow cells. Like P130, this normal cellular protein (NCP98) is a phosphoprotein which is associated in vitro with a protein kinase activity specific for tyrosyl residues. In addition, tryptic peptide analysis showed that NCP98 is structurally related to P130. The expression of NCP98 is tissue specific, and highest in bone marrow cells of the granulocytic lineage. The isolation of phenotypic revertants of rat cells transformed by FSV suggested a relationship between the expression of P130, its enzymatic activity and cellular transformation. Three types of revertants have been characterized, all of them susceptible to retransformation after superinfection with FSV. Type I revertants (3 clones) have lost the complete FSV provirus. Type II revertant (1 clone) has a mutated FSV provirus which encodes a transformation defective protein of MW 130,000. This protein has no kinase activity in vitro, is underphosphorylated and lacks phosphotyrosine in vivo. In superinfected clones, it appears to be a substrate of P140, the transforming protein of another strain of FSV used in the superinfection. The mutated protein is associated in vivo with two cellular proteins (90K and 50K) which have been implicated in the binding of the transforming proteins coded by conditional mutants of avian sarcoma viruses. (Author abstract)

78. PREFERENTIAL EXPRESSION OF THE C-FPS PROTEIN IN CHICKEN MACROPHAGES AND GRANULOCYTIC CELLS

Samarut J, Mathey-Prevot B, Hanafusa H
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Mol Cell Biol; 5(5):1067-72 1985

We have studied the expression of the protein kinase activity of NCP98, the c-fps gene product, in several hemopoietic tissues of chickens as a function of the developmental stage of these organs. We found that in bone marrow, spleen, and bursa, maximum NCP98 kinase activity on a per-cell basis correlates with the peak of granulopoiesis in these organs. Furthermore, in a bovine serum albumin density gradient fractionation of bone marrow cells, granulocytic cells appeared to account for most of the NCP98 kinase activity. No correlation was found between the distribution of erythrocytic, lymphocytic, or thrombocytic cells and the distribution of the expression of NCP98 kinase activity. However, NCP98 protein and kinase activity were 10-fold higher in macrophages than in bone marrow. In addition, depletion by complement-mediated lysis of erythrocytic cells in bone marrow did not significantly reduce the total recovery of NCP98 kinase activity. These results argue for the specific expression of the c-fps gene product in granulocytic cells and macrophages.

79. NUCLEOTIDE SEQUENCE AND TOPOGRAPHY OF CHICKEN C-FPS. GENESIS OF A RETROVIRAL ONCOGENE ENCODING A TYROSINE-SPECIFIC PROTEIN KINASE

Huang CC, Hammond C, Bishop JM
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J Mol Biol; 181(2):175-86 1985

We isolated molecular clones of chicken DNA that carry portions of the cellular proto-oncogene c-fps and then determined the nucleotide sequence of all regions of the gene that are related to the retroviral oncogene v-fps. The homology of v-fps within c-fps resides on at least 19 interspersed segments, 17 of which represent complete exons and two of which may represent only portions of exons. Fusion of these segments reconstructs a facsimile of v-fps. The arrangement of introns and exons within c-fps differs from that of the related proto-oncogene c-src in the domains of the two genes that encode tyrosine-specific protein kinase activity. It therefore appears likely that the introns arose subsequent to the gene duplication that engendered c-src and c-fps. The data also reveal potential junctions between viral and cellular domains in the genomes of two independently isolated avian sarcoma viruses (the PRCII and Fujinami strains). The lefthand junctions can be well defined: they occur at the same position in c-fps but at different positions in the viral gene gag. The righthand junctions cannot be defined as precisely because they include a sequence of 10 to 15 nucleotides whose origin is not known. In the genome of PRCII virus, the composition of this sequence suggests that it arose from the polyadenylated 3' terminus of the c-fps messenger RNA. If this deduction proves to be correct, the data will provide direct evidence that the righthand recombination during transduction by retroviruses occurs between RNA intermediates. Irrespective of these ambiguities, both junctions are located within exons of c-fps, and both may have been formed by

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non-homologous recombination (although the evidence for the latter statement is not decisive). A sequence of 1020 nucleotides has been deleted from the transduced version of c-fps in the genome of PRCII virus, apparently by homologous recombination between sequences repeated within c-fps. Fujinami virus may contain the entire coding domain of c-fps, but mutations have created 26 amino acid substitutions in the viral version of the gene. By contrast, the partially deleted version of c-fps in PRCII virus contains no mutations that would alter the amino acid sequence.

80. CELLULAR LOCALIZATION OF C-FPS GENE PRODUCT NCP98

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J Virol, 52(3):913-8, 1984

We compared the intracellular location of the product of the c-fps proto-oncogene, NCP98, with that of its viral homolog P140, the transforming protein of Fujinami sarcoma virus. Using the technique of biochemical subcellular fractionation, we determined that 60 to 90% of NCP98 and its associated kinase activity are in the soluble fraction of a chicken myeloblast cell line. This fractionation behavior differs from that of P140, which is found predominantly in the particulate fraction, both in Fujinami sarcoma virus-infected chicken embryo fibroblasts and in Fujinami sarcoma virus-infected myeloblasts. The fractionation behavior of NCP98 is, however, similar to that of the P140 encoded by a temperature-sensitive strain of Fujinami sarcoma virus in infected cells grown at the nonpermissive temperature. The absence of gag sequences from NCP98 is not responsible for the difference in fractionation behavior: the v-fps transforming protein of strain F36, P91, which lacks gag sequences, is also predominantly particulate. These results indicate that association with cellular structural components correlates with the transforming activity of proteins containing fps sequences.

81. IMMUNOLOGICAL DETECTION OF CELLULAR TARGETS FOR V-ONC GENE CODED TYROSINE KINASES

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Genetic and Phenotypic Markers of Tumors, Aaronson SA, Frati L, Verna R, eds, New York, Plenum Press, p 297-303, 1984.

Immunological detection of cellular targets for v-onc gene-coded tyrosine kinases was studied in cells transformed by Fujinami sarcoma virus (FSV) and feline sarcoma virus of either the Snyder-Theilen (ST-FeSV) or the Gardner-Arnstein strain (GA-FeSV). Onc genes of these viruses (v-fps and v-fes) code for tyrosine phosphokinases that are known to be associated with the plasma membrane and/or the cytoskeleton. Evidence is presented that azobenzyl-phosphonate (ABP) antibodies, used for immunofluorescence microscopy, decorate structures located where plasma membrane and cytoskeleton interact. Immunoprecipitation by ABP antibodies of phosphorylated detergent-insoluble proteins from the following cell sources studied: SR-BALB mouse fibroblasts, SR-A rat fibroblasts, ST-FeSV- and GA-FeSV-transformed Fisher rat embryo cells, and FSV-transformed rat fibroblasts. The ABP antibodies precipitated from each of the transformed lines a phosphotyrosyl protein of mol wt identical to that of the viral oncoprotein. p60 in cells transformed by v-src, pp140 in cells transformed by v-fps, and pp110 in cells transformed by v-fes. These data are consistent with the evidence that kinases coded by onc gene phosphorylate themselves at tyrosine. (13 Refs)

82. ANTIBODIES OF PREDETERMINED SPECIFICITY DETECT TWO RETROVIRAL ONCOGENE PRODUCTS AND INHIBIT THEIR KINASE ACTIVITIES

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Proc Natl Acad Sci USA, 80(5):1246-1250, 1983

Oligopeptides predicted from the nucleotide sequence of the oncogene v-fes of feline sarcoma virus (FeSV) were synthesized chemically and used to generate specific antibodies. Antisera against a 12-amino-acid-long oligopeptide (12-mer) located 42 residues from the carboxyl terminus of the v-fes coding sequence efficiently recognized the transforming proteins encoded by Snyder-Theilen (ST) and Gardner-Arnstein (GA) strains of FeSV. This 12-mer also contains 10 amino acid residues homologous in order and position to those predicted from the nucleotide sequence of the oncogene v-fps of avian Fujinami sarcoma virus (FSV). The anti-12-mer immunoprecipitated the FSV-specific transforming protein molecules from FSV-transformed cells. Binding of these antipeptide antibody molecules to the v-fes and the v-fps gene products inhibited their associated tyrosine-specific protein kinase activities. The ability to generate such site-specific antisera to the products of related oncogenes will be valuable in the molecular characterization of retroviral transforming proteins and their normal cellular homologs. (Author abstract) (40 Refs)

83. DETECTION AND LOCALIZATION OF A PHOSPHOTYROSINE-CONTAINING ONC GENE PRODUCT IN FELINE TUMOR CELLS

Chen AP, Essex M, de Noronha F
Hematol Blutttransfus, 28:227-35, 1983

Protein phosphorylation by a tyrosine-specific kinase is now recognized as a common event in retrovirus-transformed cells. We report in this communication that the feline sarcoma virus (FeSV) encoded transformation-specific proteins (gag-fes fusion proteins) and their associated protein kinases are also found in the FeSV in vivo induced tumor preparations, either in the form of fresh tumor homogenate or in the form of cultured cells. With the combined use of subcellular fractionation and detergent extraction we found that the protein kinase activity was present in both the membrane fraction (P100) and the cytosol (S100). The gag-fes proteins of two different strains of FeSV were found to associate with the cell framework to different degrees, suggesting that the specific conformational presentation of these proteins may be dictated by the unique portion of each polyprotein. The same gag-fes transformation related proteins could be immunoprecipitated with antiserum to phosphotyrosine.

84. HUMORAL IMMUNE RESPONSE OF TUMOR BEARING ANIMALS AND ONCOGENE PRODUCT (MEETING ABSTRACT)

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XXIIth Symposium for Comparative Research on Leukemia and Related Diseases, July 7-11, Hamburg, p 71, 1985.

Feline sarcoma virus (FeSV) is a recombinant of a cellular oncogene and helper feline leukemia virus (FeLV), and the FeSV genome consists of FeLV gag gene and a specific onc gene. In FeSV (FeLV) infected/transformed cells, a large mol wt protein carrying both gag and onc determinants is detected in addition to regular FeLV structural proteins. Cats injected with Snyder-Theilen FeSV (ST-FeSV)-transformed virus-producing cat cells had antibodies, when they rejected

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the tumor, to the transforming polyprotein p85gag-fes as well as viral antigens as determined by SDS-PAGE Western blotting analysis, while those who failed to reject the tumor had no antibody activities. In order to distinguish antiviral antibodies from anti-onc protein antibodies, rats were injected with syngeneic ST-FeSV-transformed virus-nonproducing cells. After tumor regression, antibodies reactive to p85gag-fes but not to FeLV gp70 were obtained. These antibodies, however, failed to recognize any antigen on the surface of the ST-FeSV-transformed cells in surface immuno-fluorescence test or lactoperoxidase I-125 labeling immunoprecipitation. A monoclonal antibody to a fes determinant of p85 did not immunoprecipitate any surface protein from the transformed cells, while it immunoprecipitated p85 metabolically labeled with S-35 methionine. Immunoperoxidase staining of the transformed cells with this monoclonal antibody demonstrated cytoplasmic localization of the oncogene product. These results suggested that anti-onc protein antibodies found in ST-FeSV tumor regressor animals are not the primary cause but a result of the tumor regression.

85. NUCLEOTIDE SEQUENCES OF FELINE RETROVIRAL ONCOGENES (V-FES) PROVIDE EVIDENCE FOR A FAMILY OF TYROSINE-SPECIFIC PROTEIN KINASE GENES

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Cell; 30(3):775-785 1982

The nucleotide sequences encoding the transforming polyproteins of the Snyder-Theilen and Gardner-Arnstein strains of feline sarcoma virus (FeSV) have been determined. These sequences include a viral transforming gene (v-fes), derived from cellular proto-oncogene sequences (c-fes) of domestic cats by recombination with feline leukemia virus (FeLV). The v-fes sequences are predicted to encode a polypeptide domain strikingly similar to that specified by the transforming gene (v-fps) of the avian Fujinami sarcoma virus. In addition, the 3' 0.8 kilobase pairs of v-fes encode amino acid sequences homologous to the carboxy-terminal portion of pp60src, the transforming protein encoded by the avian Rous sarcoma virus src gene. Thus different feline and avian retroviral transforming genes, all of which encode functionally related proteins with associated tyrosine-specific kinase activities, must be derived from divergent members of the same proto-oncogene family. (Author abstract) (71 Refs)

6.

fms protein product

86. THE PRODUCT OF THE C-FMS PROTO-ONCOGENE: A GLYCOPROTEIN WITH ASSOCIATED TYROSINE KINASE ACTIVITY

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Science; 228(4697):320-2 1985

The c-fms proto-oncogene is a member of a gene family that has been implicated in tumorigenesis. Glycoproteins encoded by c-fms were identified in cat spleen cells by means of an immune-complex kinase assay performed with monoclonal antibodies to v-fms-coded epitopes. The major form of the normal cellular glycoprotein has an apparent molecular weight of 170,000 and, like the product of the viral oncogene, serves as a substrate for an associated tyrosine-specific protein kinase activity in vitro. The results suggest that the transforming glycoprotein specified by v-fms is a truncated form of a c-fms-coded growth factor receptor.

87. THE C-FMS PROTO-ONCOGENE PRODUCT IS RELATED TO THE RECEPTOR FOR THE MONONUCLEAR PHAGOCYTE GROWTH FACTOR, CSF-1

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Cell; 41(3):665-76 1985

The feline c-fms proto-oncogene product is a 170 kd glycoprotein with associated tyrosine kinase activity. This glycoprotein was expressed on mature cat macrophages from peritoneal inflammatory exudates and spleen. Similarly, the receptor for the murine colony-stimulating factor, CSF-1, is restricted to cells of the mononuclear phagocytic lineage and is a 165 kd glycoprotein with an associated tyrosine kinase. Rabbit antisera to a recombinant v-fms-coded polypeptide precipitated the feline c-fms product and specifically cross-reacted with a 165 kd glycoprotein from mouse macrophages. This putative product of the murine c-fms gene exhibited an associated tyrosine kinase activity in immune complexes, specifically bound murine CSF-1, and, in the presence of the growth factor, was phosphorylated on tyrosine in membrane preparations. The murine c-fms proto-oncogene product and the CSF-1 receptor are therefore related, and possibly identical, molecules.

88. STRUCTURAL ALTERATION OF VIRAL HOMOLOGUE OF RECEPTOR PROTO-ONCOGENE FMS AT CARBOXYL TERMINUS

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Nature; 320(6059):277-80 1986

A role for proto-oncogenes in the regulation and modulation of cell proliferation has been suggested by the findings that the B-chain of platelet-derived growth factor (PDGF) is encoded by the proto-oncogene sis and that the erb-B oncogene product is a truncated form of the epidermal growth factor (EGF) receptor. Furthermore, the product of the proto-oncogene fms (c-fms) may be related or identical to the receptor for macrophage colony-stimulating factor (CSF-1). v-fms is the transforming gene of the McDonough strain of feline sarcoma virus (SM-FeSV) and belongs to the family of src-related oncogenes which have tyrosine-specific kinase activity. Furthermore, nucleotide sequence analysis of the v-fms gene product revealed topological properties of a cell-surface receptor protein. To elucidate the features involved in the conversion of a normal cell-surface receptor gene into an oncogenic one, we have now determined the complete nucleotide sequence of a human c-fms complementary DNA. The 972-amino-acid c-fms protein has an extracellular domain, a membrane-spanning region, and a cytoplasmic tyrosine protein kinase domain. Comparison of the feline v-fms and human c-fms sequences reveals that the proteins share extensive homology but have different carboxyl termini.

89. THE FMS ONCOGENE (MEETING ABSTRACT)

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Fed Proc; 44(3):549 1985

The retroviral oncogene v-fms of the McDonough strain of feline sarcoma virus (SM-FeSV) encodes an integral transmembrane glycoprotein whose expression at the cell surface is required for transformation. Two hydrophobic 'signal' peptides orient the nascent glycoprotein in membranes of the

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endoplasmic reticulum (ER) with its glycosylated aminoterminus (ca 450 amino acids) in the ER cisternae and its carboxylterminal portion (ca 400 amino acids) in the cytoplasm. The distal cytoplasmic domain shows amino acid sequence homology to tyrosine-specific protein kinases and appears to be responsible for an associated in vitro kinase activity. Transport to the cell surface places the aminoterminal portion outside the cell and the cytoplasmic kinase domain at the inner surface of the plasma membrane. Monoclonal antibodies to v-fms-coded aminoterminal epitopes reacted with live SM-FeSV infected cells and were used to isolate cells of the transformed phenotype by fluorescence-activated flow cytometry. Certain of these reagents reacted with a normal cellular protein presumed to be the product of the c-fms protooncogene. Based on biochemical and topological considerations, the c-fms product is predicted to be a cell surface receptor for a growth factor.

90. CELL SURFACE EXPRESSION OF V-FMS-CODED GLYCOPROTEINS IS REQUIRED FOR TRANSFORMATION

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Mol Cell Biol; 4(10):1999-2009 1984

The viral oncogene v-fms encodes a transforming glycoprotein with in vitro tyrosine-specific protein kinase activity. Although most v-fms-coded molecules remain internally sequestered in transformed cells, a minor population of molecules is transported to the cell surface. An engineered deletion mutant lacking 348 base pairs of the 3.0-kilobase-pair v-fms gene encoded a polypeptide that was 15 kilodaltons smaller than the wild-type v-fms gene product. The in-frame deletion of 116 amino acids was adjacent to the transmembrane anchor peptide located near the middle of the predicted protein sequence and 432 amino acids from the carboxyl terminus. The mutant polypeptide acquired N-linked oligosaccharide chains, was proteolytically processed in a manner similar to the wild-type glycoprotein, an d exhibited an associated tyrosine-specific protein kinase activity in vitro. However, the N-linked oligosaccharides of the mutant glycoprotein were not processed to complex carbohydrate chains, and the glycoprotein was not detected at the cell surface. Cells expressing high levels of the mutant glycoprotein did not undergo morphological transformation and did not form colonies in semisolid medium. The transforming activity of the v-fms gene product therefore appears to be mediated through target molecules on the plasma membrane.

91. SUBCELLULAR LOCALIZATION OF GLYCOPROTEINS ENCODED BY THE VIRAL ONCOGENE V-FMS

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J Virol; 51(3):730-41 1984

The McDonough strain of feline sarcoma virus encodes a polyprotein that is cotranslationally glycosylated and proteolytically cleaved to yield transforming glycoproteins specified by the viral oncogene v-fms. The major form of the glycoprotein (gp120fms) contains endoglycosidase H-sensitive, N-linked oligosaccharide chains lacking fucose and sialic acid, characteristic of glycoproteins in the endoplasmic reticulum. Kinetic and steady-state measurements showed that most gp120fms molecules were not converted to mature forms containing complex carbohydrate moieties. Fixed-cell immunofluorescence confirmed that the majority of v-fms-coded antigens were internally sequestered in transformed cells. Dual-antibody fluorescence performed with antibodies to intermediate filaments (IFs) showed that the IFs of transformed cells were rearranged, and their distribution coincided with that of v-fms-coded antigens. No specific disruption of actin cables was observed. The v-fms gene products cofractionated with IFs isolated from virus-transformed cells and reassociated with IFs self-assembled in vitro. A minor population of v-fms-coded molecules (gp140fms) acquired endoglycosidase H-resistant, N-linked oligosaccharide chains containing fucose and sialic acid residues, characteristic of molecules processed in the Golgi complex. Some gp140fms molecules were detected at the plasma membrane and were radiolabeled by lactoperoxidase-catalyzed iodination of live transformed cells. We suggest that v-fms-coded molecules are translated as integral transmembrane glycoproteins, most of which are inhibited in transport through the Golgi complex to the plasma membrane.

92. NUCLEOTIDE SEQUENCE OF THE FELINE RETROVIRAL ONCOGENE V-FMS SHOWS UNEXPECTED HOMOLOGY WITH ONCOGENES ENCODING TYROSINE-SPECIFIC PROTEIN KINASES

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Proc Natl Acad Sci USA; 81(1):85-9 1984

The nucleotide sequence encoding the transforming polyprotein of the McDonough strain of feline sarc oma virus was determined. This sequence includes 231 nucleotides specifying a leader peptide, 1,377 nucleotides encoding most of the feline leukemia virus-derived gag gene, and 2,969 nucleotides representing the viral transforming gene v-fms. A single open reading frame was predicted to encode a fusion polyprotein of 160,000 daltons (P160gag-fms). Fourteen potential sites for glycosylation were predicted within the v-fms-encoded portion of the protein, consistent with previous observations that the primary translation product is rapidly glycosylated. The presence of hydrophobic signal peptides within the amino-terminal leader sequence and in the middle of the v-fms-encoded moiety suggests that the transforming glycoprotein becomes oriented with its amino terminus within the lumen of the rough endoplasmic reticulum and its carboxyl terminus protruding across the membrane of the rough endoplasmic reticulum into the cytoplasm. The latter portion of the protein shows unexpected homology to tyrosine-specific protein kinases encoded by several of the known retroviral oncogenes.

7.

fgr protein product

93. ISOLATION AND CHROMOSOMAL LOCALIZATION OF THE HUMAN FGR PROTOONCOGENE, A DISTINCT MEMBER OF THE TYROSINE KINASE GENE FAMILY

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Proc Natl Acad Sci USA; 82(19):6595-9 1985

The cell-derived domain of Gardner-Rasheed feline sarcoma virus (GR-FeSV) consists of a gamma-actin- and a tyrosine-specific protein kinase-encoding sequence designated v-fgr. By utilizing a v-fgr probe, it was possible to detect related sequences present at low copy number in DNAs of a variety of mammalian species and to isolate a human fgr homologue. Comparative studies revealed that this human DNA clone represented all but 200 base pairs of v-fgr. Analysis of human genomic DNA demonstrated that the fgr protooncogene was distinct from the cellular homologues of other retrovirus onc

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genes. In addition, the fgr protooncogene was localized to the distal portion of the short arm of human chromosome 1 at p36.1-36.2 by in situ hybridization. Taken together, our findings establish that the fgr protooncogene is a unique member of the tyrosine kinase gene family.

94. GENE PRODUCT OF V-FGR ONC: HYBRID PROTEIN CONTAINING A PORTION OF ACTIN AND A TYROSINE-SPECIFIC PROTEIN KINASE

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Science; 223(4631):63-6 1984

The nucleotide sequence of the region of Gardner-Rasheed feline sarcoma virus (GR-FeSV) encoding its primary translation product, p70gag-fgr, has been determined. From the nucleotide sequence, the amino acid sequence of this transforming protein was deduced. Computer analysis indicates that a portion of P70gag-fgr has extensive amino acid sequence homology with actin, a eukaryotic cytoskeletal protein. A second region of P70gag-fgr is closely related to the tyrosine-specific kinase gene family. Thus, the v-fgr oncogene appears to have arisen as a result of recombinational events involving two distinct cellular genes, one coding for a structural protein and the other for a protein kinase.

95. MOLECULAR CLONING OF INTEGRATED GARDNER-RASHEED FELINE SARCOMA VIRUS: GENETIC STRUCTURE OF ITS CELL-DERIVED SEQUENCE DIFFERS FROM THAT OF OTHER TYROSINE KINASE-CODING ONC GENES

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J Virol; 47(3):611-9 1983

Gardner-Rasheed feline sarcoma virus (GR-FeSV) is an acute transforming retrovirus which encodes a gag-onc polyprotein possessing an associated tyrosine kinase activity. The integrated form of this virus, isolated in the Charon 21A strain of bacteriophage lambda, demonstrated an ability to transform NIH/3T3 cells at high efficiency upon transfection. Foci induced by GR-FeSV DNA contained rescuable sarcoma virus and expressed GR-P70, the major GR-FeSV translational product. The localization of long-terminal repeats within the DNA clone made it possible to establish the length of the GR-FeSV provirus as 4.6 kilobase pairs. The analysis of heteroduplexes formed between lambda feline leukemia virus (FeLV) and lambda GR-FeSV DNAs revealed the presence of a 1,700-base-pair FeLV unrelated segment, designated v-fgr, within the GR-FeSV genome. The size of this region was sufficient to encode a protein of approximately 58,000 daltons and was localized immediately downstream of the FeLV gag gene coding sequences present in GR-FeSV. Thus, it is likely that this 1.7-kilobase-pair stretch encodes the onc moiety of GR-P70. Utilizing probes representing v-fgr, we detected homologous sequences in the DNAs of diverse vertebrate species, implying that v-fgr originated from a well-conserved cellular gene. The number of cellular DNA fragments hybridized by v-fgr-derived probes indicated either that proto-fgr is distributed over a very large region of cellular DNA or represents a family of related genes. By molecular hybridization, v-fgr was not directly related to the onc genes of other known retroviruses having associated tyrosine kinase activity.

96. HUMAN C-F GR GENE DOES NOT CONTAIN CODING SEQUENCE FOR ACTIN-LIKE PROTEIN

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Gann; 76(3):155-9 1985

f gr is a member of the protein kinase oncogene family and was found to have the highest homology to yes gene among the members of the family. Using a v-yes probe, a molecular clone of human c-f gr locus was isolated from a genomic library. Nucleotide sequence determination revealed that the actin g ene-like sequence found in v-f gr gene was absent at the corresponding position of the c-f gr gene. Thus, the v-f gr gene product is considered to be a tri-chimeric gene product consisting of viral gag gene and two distinct cellular genes, actin gene and the proto-f gr gene.

8.

mos protein product

97. USE OF SITE-SPECIFIC ANTIPEPTIDE ANTIBODIES TO PERTURB THE SERINE KINASE CATALYTIC ACTIVITY OF P37MOS

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J Virol; 55(3):874-6 1985

The mos oncogene of Moloney murine sarcoma virus encodes a protein of approximately 37,000 daltons (designated p37mos). We have detected a serine protein kinase activity which is closely associated with p37mos in immune complexes obtained with antibodies [anti-mos(37-55) serum] that were generated with a peptide containing amino acids 37 through 55 of the v-mos protein (S. A. Maxwell and R. B. Arlinghaus, Virology 143:321-333, 1985). Immune complexes that were derived with antibodies generated against peptides representing the C-terminal 8 or 12 amino acids of v-mos (anti-C2 and anti-C3 serum, respectively) exhibited very little kinase activity capable of phosphorylating p37mos. Treatment of anti-mos(37-55) complexes containing active v-mos kinase with anti-C3 or anti-C2 serum resulted in a dramatic reduction of the in vitro phosphorylation of p37mos. Antiserum blocked with the appropriate C-terminal peptide had no inhibitory effect on the phosphorylation of p37mos in anti-mos(37-55) complexes which indicated that the inhibition of v-mos kinase activity was a specific effect of these antibodies. The specific inhibition of the in vitro phosphorylation of p37mos by antibodies directed against the C terminus of the v-mos protein provides strong evidence that the v-mos gene encodes a serine protein kinase. In addition, the extreme C terminus of p37mos may be critical for an active v-mos kinase.

98. COMPLETE C-MOS (RAT) NUCLEOTIDE SEQUENCE: PRESENCE OF CONSERVED DOMAINS IN C-MOS PROTEINS

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Nucleic Acids Res; 12(4):2147-56 1984

Recently we described the isolation of c-mos (rat). The gene belongs to the family of oncogenes. Some facts render c-mos unique among the oncogenes: a) it does not contain intervening sequences and b) its expression was never detected in a large number of normal mouse tissues examined. We undertook the sequence analysis of c-mos (rat) in order to compare it to the nucleotide sequences published for c-mos (mouse), c-mos (human), c-src and bovine protein kinase. C-mos (rat) contains an open reading frame of 1017 nucleo-

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tides, coding for a polypeptide of 339 amino acids. C-mos (rat)-makes use of the same ATG that defines the N-terminus of the c-mos (human) protein. By comparing all c-mos sequences available we found sequences with high mutational rates to be confined to certain domains. This comparison, together with data on the biological activities of the cloned DNA, allowed us to tentatively define regions involved in (a) function(s) of c-mos other than transformation.

99. EPIDERMAL GROWTH FACTOR PRECURSOR IS RELATED TO THE TRANSLATION PRODUCT OF THE MOLONEY SARCOMA VIRUS ONCOGENE MOS

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Proc Natl Acad Sci USA; 82(7):1921-5 1985

Murine epidermal growth factor (EGF) is synthesized as part of a large precursor (pro-EGF), which is thought to span the cell membrane. Comparison of the published pro-EGF sequence with the sequences of the translation products of viral oncogenes reveals that pro-EGF is related to the translation product of mos, the oncogene of Moloney murine sarcoma virus. Similarity is greatest between the COOH-terminal region of v-mos (residues 317-360) and part of the cytoplasmic domain of pro-EGF (residues 1127-1174). Statistical comparison of these sequences indicates that the probability of the similarity arising by chance is less than 2 X 10(-8). This similarity extends to the corresponding regions of the translation products of the cellular homologues (c-mos) of the v-mos gene present in normal murine and human DNA. Similarities are also observed between two other regions of the murine c-mos sequence (residues 48-134 and 196-275) and parts of the extracellular domain of pro-EGF (residues 565-651 and 741-817, respectively). All three mos genes are members of the tyrosine kinase family of oncogenes, as is erbB, the oncogene of avian erythroblastosis virus. Since the sequences of the erbB translation product and the EGF receptor are closely related, the relationship between mos and pro-EGF suggests that pro-EGF and the EGF receptor have evolved from a common ancestor.

100. MOLECULAR BASIS UNDERLYING PHENOTYPIC REVERTANTS OF MOLONEY MURINE SARCOMA VIRUS MUSVTS110

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J Virol; 57(1):310-7 1986

We examined the molecular basis for phenotypic reversion in cells infected with a transformation mutant of murine sarcoma virus, MuSVts110. In MuSVts110-infected NRK cells (6m2 cells), the manifestation of the transformed phenotype at 33 degrees C and the normal phenotype at 39 degrees C is governed by thermosensitive splicing of the MuSVts110 primary transcript, a 4.0-kilobase (kb) RNA which contains the gag and mos genes joined out of frame. At 33 degrees C, selectively, the 4.0-kb RNA is processed to a spliced 3.5-kb RNA in which the gag and mos genes are rejoined in a continuous open readi ng frame, thus allowing synthesis of the P85gag-mos-transforming protein. In contrast, the MuSVts110 revertant cell lines (designated 54-5A4 and 204-3) appear transformed at all growth temperatures from 33 to 39 degrees C and express a P100gag-mos-transforming protein from an apparently unprocessed 4.0-kb viral RNA. In the current study we established both by S1 nuclease analysis and primer extension sequencing that the revertant 54-5A4 and 204-3 4.0-kb viral RNAs suffered a 5-base deletion at the intron-exon border of the 3' splice site. The effect of this deletion is twofold. First, because of the damage to the 3' splice site, the revertant viral 4.0-kb RNAs cannot be processed to the spliced 3.5-kb RNA and, consequently, cannot be translated to P85gag-mos. Second, the 5-base deletion excises an in-frame stop codon positioned at the intron-exon border in the parental RNA and restores the original mos gene reading frame. The net effect is to produce a continuous open reading frame from the gag, alternate mos, and authentic mos gene reading frames which are fused together in the revertant 4.0-kb RNA. This continuous open reading frame can be translated into the P100gag-mos-transforming protein at any growth temperature.

9.

abl protein product

101. AN ALTERATION OF THE HUMAN C-ABL PROTEIN IN K562 LEUKEMIA CELLS UNMASKS ASSOCIATED TYROSINE KINASE ACTIVITY

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Cell; 37(3):1035-42 1984

The v-abl protein is known to be a tyrosine-specific protein kinase. However, its normal cellular homolog, c-abl P150, is not detectably phosphorylated on tyrosine in vivo or in vitro. The lack of associated tyrosine kinase activity for the c-abl protein seems paradoxical since it is the c-abl-derived sequences of the v-abl protein that encode the kinase activity. We have detected an altered human c-abl protein (P210) with associated tyrosine kinase activity in the K562 leukemia cell line. K562 cells are known to have a 9:22 chromosomal translocation involving the c-abl locus and have amplified the c-able gene 4 to 8 fold. The altered P210 human c-abl is serologically and structurally related to the normal c-abl protein. A structural alteration of the human c-abl protein. K562 cells may have unmasked its associated tyrosine kinase activity. This altered c-abl protein may have important implications for a mechanism of activation of this oncogene.

102. THE HUMAN CELLULAR ABL GENE PRODUCT IN THE CHRONIC MYELOGENOUS LEUKEMIA CELL LINE K562 HAS AN ASSOCIATED TYROSINE PROTEIN KINASE ACTIVITY

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Oncology Section, Johnson and Johnson Biotechnology Center, La Jolla, California.
Virology; 140(2):230-8 1985

Three antisera against the mouse v-abl gene product were used to identify two potential human c-abl gene products in the chronic myelogenous leukemia cell line K562. Two antipeptide sera were generated in rabbits using the predicted amino acid sequence of the mouse v-abl gene product. One antiserum was made against a polypeptide overlapping the in vivo tyrosine phosphorylation site of murine P120gag-abl and what is believed to be a homologous tyrosine phosphorylation site of the predicted normal human c-abl gene product (v-abl 263-280). The second antipeptide serum, abl 389-403, was generated against a predicted hydrophilic peptide of the v-abl gene product. Immunoprecipitation from K562 cells metabolically labeled with [32P]orthophosphate by a mouse tumor regressor and abl 389-403 antipeptide sera detected two proteins of 190,000 and 240,000 Da. Both proteins were labeled primarily at serine and, to a much lesser extent, at tyrosine residues. Immune complex kinase assays using conditions that allow the tyrosine phosphorylation of P120gag-abl showed that in vitro phosphorylation of P190 and P240 occurs primarily at tyrosine residues. The detection of these enzymatically active human c-abl gene products is a rare ob-

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servation which may be in part attributed to the c-abl gene translocation from chromosomes 9 to 22 occurring in the vast majority of chronic myelogenous leukemia patients.

103. CELL LINES AND CLINICAL ISOLATES DERIVED FROM PH1-POSITIVE CHRONIC MYELOGENOUS LEUKEMIA PATIENTS EXPRESS C-ABL PROTEINS WITH A COMMON STRUCTURAL ALTERATION

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Proc Natl Acad Sci USA: 82(6):1810-4 1985

The Philadelphia chromosome (Ph1), observed in greater than 90% of chronic myelogenous leukemia (CML) patients, results from a specific chromosomal translocation involving the c-abl gene. The translocation breakpoint occurs near c-abl and correlates with the production of an altered c-abl mRNA. In the CML-derived cell line K562, Ph1 is accompanied by a structurally altered c-abl protein (P210c-abl) with in vitro tyrosine kinase activity not detected with the normal c-abl protein (P145c-abl). We have examined c-abl proteins in other Ph1-positive CML cell lines and found that they all express P210c-abl. P210c-abl was also detected in bone marrow cells from CML patients with Ph1 in the accelerated and blast crisis phases of the disease. Comparison of the [35S]methionine-labeled tryptic peptides generated from the normal P145c-abl and P210c-abl showed that they have closely related structures, but additional polypeptide sequences are present in P210c-abl. Based on these results we propose that translocation of c-abl in Ph1-positive CML results in the creation of a chimeric gene leading to the production of a structurally altered c-abl protein with activated tyrosine kinase activity. The altered P210 c-abl protein is strongly implicated in the pathogenesis of CML.

104. ACTIVATION OF THE C-ABL ONCOGENE BY VIRAL TRANSDUCTION OR CHROMOSOMAL TRANSLOCATION GENERATES ALTERED C-ABL PROTEINS WITH SIMILAR IN VITRO KINASE PROPERTIES

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Mol Cell Biol: 5(1):204-13 1985

The v-abl protein of Abelson murine leukemia virus is a tyrosine-specific kinase. Its normal cellular homolog, murine c-abl, does not possess detectable tyrosine kinase activity in vitro. Previously, we have detected tyrosine kinase activity in vitro for an altered c-abl gene product (c-abl P210) in the K562 human chronic myelogenous leukemia cell line. The expression of this variant c-abl gene product correlates with chromosomal translocation and amplification of the c-abl gene in K562 cells. Like v-abl, c-abl P210 is a fusion protein containing non-abl sequences near the amino terminus of c-abl. We compared the in vitro tyrosine kinase activity of c-abl P210 with that of wild-type murine v-abl. The remarkable similarities of these two proteins with respect to cis-acting autophosphorylation, trans-acting phosphorylation of exogenous substrates, and kinase inhibition, using site-directed abl-specific antisera, suggested that c-abl P210 could function similarly to v-abl in vivo. In addition, c-abl P210 possessed an associated serine kinase activity in immunoprecipitates. The serine kinase activity was not inhibited by site-directed, abl-specific antisera that inhibit the tyrosine kinase activity, suggesting that the serine kinase activity is not an intrinsic property of c-abl P210. Thus, the activation of the c-abl gene in a human leukemia cell line may have functional consequences analogous to activation of the c-abl gene in Abelson murine leukemia virus.

105. MONOCLONAL ANTIBODIES SPECIFIC FOR V-ABL- AND C-ABL-ENCODED MOLECULES

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J Virol: 57(3):1182-6 1986

Monoclonal antibodies specific for regions of the transforming protein of Abelson murine leukemia virus were prepared. Antibodies directed against the kinase domain inhibited the autophosphorylation of v-abl proteins, and all of the antibodies reacted with the products of the murine and human c-abl loci.

106. HOMOLOGY BETWEEN PHOSPHOTYROSINE ACCEPTOR SITE OF HUMAN C-ABL AND VIRAL ONCOGENE PRODUCTS

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Nature: 304(5922):167-9 1983

The human homologues of several independent viral oncogenes, each of which encodes tyrosine-specific protein kinases, have been identified. Of these, three (v-src, v-yes and v-fes/fps) are known to exhibit considerable sequence homology, particularly in the regions of their phosphorylation acceptor sites. In the present study, sequences encoding the tyrosine phosphorylation acceptor sites of the Abelson murine leukaemia virus oncogene, v-abl, and its human cellular homologue, c-abl, have been identified and their nucleic acid sequences determined. Our results establish extensive homology between this region of c-abl and acceptor domains of the v-src, v-yes and v-fes/fps family of viral oncogenes, as well as more distant relatedness to the catalytic chain of the mammalian cyclic AMP-dependent protein kinase. These findings suggest that, of the homologues of retroviral oncogenes with tyrosine protein kinase activity examined to date, all were probably derived from a common progenitor and may represent members of a diverse family of cellular protein kinases.

107. THE MINIMUM TRANSFORMING REGION OF V-ABL IS THE SEGMENT ENCODING PROTEIN-TYROSINE KINASE

Prywes R. Foulkes JG. Baltimore D
Whitehead Institute for Biomedical Research. Cambridge. Massachusetts.
J Virol: 53(1):114-22 1985

Only 1.2 kilobases (kb) at the 5' end of the 3.9-kb v-abl sequence in Abelson murine leukemia virus is required for fibroblast transformation. A precise delineation of this minimum transforming region was made by using small 5' or 3' deletions. Insertions of four amino acids, generated by putting synthetic DNA linkers into various restriction enzyme cleavage sites, abolished transforming activity, indicating that much of the internal sequence of the minimum transforming region plays a critical role in the transformation process. This 5' 1.2 kb of v-abl encodes protein-tyrosine kinase activity when expressed in Escherichia coli. Each of the mutations which caused a loss of transformation activity also resulted in a loss of protein-tyrosine kinase activity when expressed in E. coli. The minimum transforming region of v-abl contains amino acid homology to other protein-tyrosine kinase oncogenes, and a comparison with these oncogenes is presented.

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108. PROTEIN PHOSPHORYLATION AT TYROSINE RESIDUES IN V-ABL TRANSFORMED MOUSE LYMPHOCYTES AND FIBROBLASTS

Saggioro D, Ferracini R, Di Renzo MF, Naldini L, Chieco-Bianchi L, Comoglio PM
Laboratory of Oncology, University of Padua, Italy.
Int J Cancer; 37(4):623-6 1986

Phosphotyrosine antibodies were employed to immunodecorate and immunoprecipitate proteins phosphorylated at tyrosine residues in cells transformed by Abelson murine leukemia virus (A-MuLV). In pre-B and pre-T lymphoma cells transformed by A-MuLV, the major phosphotyrosine-containing protein has an MW of 160 kDa and shares immunologically detectable sequences with the v-abl oncogene product. Moreover, two different proteins of approximately 100 and 68 kDa, heavily phosphorylated at tyrosine, were identified. Lack of immunological cross-reactivity with viral products and phosphopeptide mapping s howed that the 100 and 68 kDa proteins are coded by cellular genes. Phosphoproteins were undetectable in control resting lymphocytes. The 68 and the 100 kDa proteins were phosphorylated to different extents in proliferating lymphocytes, either stimulated by the growth factor IL-2, or transformed by M-MuLV (lacking the oncogene coded kinase). In fibroblasts transformed by A-MuLV, phosphotyrosine antibodies identified 2 proteins of 120 and 70 kDa. By immunological cross-reaction and by phosphopeptide mapping, the first was identified as a 120 kDa form of the v-abl coded kinase. The 70 kDa protein is coded by a cellular gene, is not structurally related to the 120 kDa v-abl kinase, and is different from any phosphotyrosine-containing protein detected in A-MuLV-transformed lymphocytes. These data show that, upon v-abl-induced transformation, phosphorylation at tyrosine takes place also on proteins other than the 160 or 120 kDa oncogene products. In lymphocytes and fibroblasts these proteins are different, suggesting that the cascade of events triggered by the v-abl gene in different cell types involves tyrosine phosphorylation of different specific proteins.

109. LOCALIZATION OF TYROSINE KINASE-CODING REGION IN V-ABL ONCOGENE BY THE EXPRESSION OF V-ABL-ENCODED PROTEINS IN BACTERIA

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J Biol Chem; 260(1):64-71 1985

A series of plasmids containing different segments of the v-abl oncogene have been constructed to express different portions of the v-abl protein in bacteria. The tyrosine kinase activity of these proteins was determined by an in vitro assay employing histones or angiotensin II as substrates for the v-abl-encoded tyrosine kinase. These experiments show that the 5'-1.2 kilobases of v-abl is necessary and sufficient to produce an active tyrosine kinase which is functional as a monomeric soluble protein. The kinase-coding region corresponds to the minimal region of v-abl required for the transformation of fibroblasts. The kinase-coding region also coincides with the conserved protein sequences which are found in other tyrosine kinases. A compact domain of the v-abl protein including this kinase-coding region can accumulate to high levels in bacteria. The C-terminal region of the v-abl protein is not needed for the kinase activity and is rapidly degraded in bacteria.

110. STRUCTURE AND FUNCTION OF THE ABELSON MURINE LEUKEMIA VIRUS TRANSFORMING GENE

Wang JY, Prywes R, Baltimore D
Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge.
Prog Clin Biol Res; 119:57-63 1983

The oncogene (v-abl) of A-MuLV is a fragment of a normal mouse gene. Only 1.2 kb on the 5'-end of the v-abl is sufficient to encode a tyrosine-specific protein kinase whose activity is important in tr ansformation. This A-MuLV protein kinase can be active in E. coli and it phosphorylates bacterial proteins. The lack of discrimination in its selection for substrates is puzzling. Because of the demonstration of such promiscuity, the identification of physiologically-relevant phosphorylation events must rely on evidence of functional modification rather than physical modification of proteins.

111. NUCLEOTIDE SEQUENCE OF ABELSON MURINE LEUKEMIA VIRUS GENOME: STRUCTURAL SIMILARITY OF ITS TRANSFORMING GENE PRODUCT TO OTHER ONCOGENE PRODUCTS WITH TYROSINE-SPECIFIC KINASE ACTIVITY

Reddy EP, Smith MJ, Srinivasan A
Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland.
Proc Natl Acad Sci USA; 80(12):3623-7 1983

The nucleotide sequence of the proviral genome of Abelson murine leukemia virus (A-MuLV), an acute transforming virus of murine origin, has been determined. Like other transforming viruses, A-MuLV contains sequences derived from its helper virus, Moloney murine leukemia virus (M-MuLV), and a cell-derived protooncogene (abl) insertion sequence. By comparison of the A-MuLV sequence with that of M-MuLV, it was possible to precisely localize and define sequences contributed by the host cellular DNA. From the nucleotide sequence, we have predicted the amino acid sequence of p120gag-abl, the product of the A-MuLV gag-abl hybrid gene. The amino acid sequence of the putative abl gene, when compared with the sequences of other tyrosine-specific protein kinases (src, fes, fps, and yes), revealed significant homologies, indicating that all these functionally related transforming genes are derived from divergent members of the same protooncogene family. In addition to the gag-abl sequence, the proviral genome was found to contain an additional open reading frame that could code for an 18,000-dalton protein, whose role is at present undetermined.

112. ISOLATION AND CHARACTERIZATION OF CAENORHABDITIS ELEGANS DNA SEQUENCES HOMOLOGOUS TO THE V-ABL ONCOGENE

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Department of Biology, University of Utah, Salt Lake City 84112.
Proc Natl Acad Sci USA; 83(7):2172-6 1986

DNA sequences homologous to the v-abl oncogene were isolated from a Caenorhabditis elegans genomic library by their ability to hybridize with a v-src probe. The DNA sequence of 2465 nucleotides of one clone was determined. This region corresponds to the 5' protein kinase domain of v-abl plus approximately equal to 375 base pairs toward the 3' end. Four potential introns were identified. The homology between the deduced amino acid sequence of the C. elegans clone and that of the 1.2-kilobase-pair protein kinase region of v-abl is 62%. The tyrosine residue corresponding to the tyrosine that is phosphorylated in the v-src protein is conserved in the C. elegans sequence. When 95 amino acids around this tyrosine were compared with the corresponding sequences of Drosophila c-abl, v-abl, and v-src, the identities were 83%, 79%, and 56%, respectively. Hybridization of the

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cloned DNA with C. elegans poly(A)+ RNA revealed a major transcript of 4.4 kilobases.

113. SPECIFIC TRANSFORMING POTENTIAL OF ONCOGENES ENCODING PROTEIN-TYROSINE KINASES

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EMBO J; 4(7):1769-74 1985

Several chimeric murine retroviruses were constructed to test whether the gag sequence of Abelson murine leukemia virus (A-MuLV) could influence the in vitro specificity of two sarcoma-inducing oncogenes: src of Rous sarcoma virus and fps of Fujinami sarcoma virus. Although the src- or fps- containing chimerae could transform fibroblasts, they were unable to mimic the action of A-MuLV in causing lymphoid transformation in vitro. A-MuLV-derived gag sequences could, however, functionally replace the 5' end of src and restore the transformation potential of a 5'-truncated src gene. To investigate this functional similarity, we replaced the gag sequence of an A-MuLV virus with the 5' end of src. This recombinant virus behaved like the A-MuLV virus from which it was derived: it transformed both fibroblasts and lymphoid cells in vitro. Taken together, these results suggest that lymphoid transformation in vitro is a specific property of abl and not of src or fps. Furthermore, it shows that a functional homology exists between the gag sequence of A-MuLV and the 5' end of src.

114. V-ABL ACTIVATES EMBRYONIC GLOBIN GENE EXPRESSION IN MOUSE ERYTHROLEUKEMIA CELLS

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Proc Natl Acad Sci USA; 83(7):2042-6 1986

The Philadelphia chromosome translocation, which is present in 90-95% of chronic myelogenous leukemia patients, involves translocation of the c-abl protooncogene to chromosome 22 and is accompanied by activation of embryonic globin gene expression in the K562 chronic myelogenous leukemia cell line. To test directly if the protein products of the translocated c-abl protooncogene can activate embryonic globin gene expression, we transfected the v-abl oncogene (which shares the property of autophosphorylation with the translocated c-abl protooncogene) into mouse erythroleukemia cells. v-abl-transfected mouse erythroleukemia cells, which contained multiple copies of the v-abl transgenome, exhibited activation of mouse embryonic globin gene expression. These results suggest that the translocated c-abl protooncogene of the Philadelphia chromosome translocation is central to the pathogenesis of chronic myelogenous leukemia and that it may result in the activation of embryonic globin genes in some chronic myelogenous leukemia cell lines.

10.

mil/raf protein product

115. SERINE- AND THREONINE-SPECIFIC PROTEIN KINASE ACTIVITIES OF PURIFIED GAG-MIL AND GAG-RAF PROTEINS

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Nature; 312(5994):558-61 1984

Retroviruses carry cell-derived oncogenes (v-onc) that have the potential to transform cells in culture and induce tumours in vivo. One of the few carcinoma-inducing viruses is the acutely transforming retrovirus MH2, which carries the putative oncogene v-mil and the known oncogene v-myc. Recently, a high degree of homology was discovered between v-mil and v-raf, the transforming gene of the murine retrovirus 3611 murine sarcoma virus (MSV), whereas homology to v-src is low. Both viruses express their oncogenes as the gag-fusion polyproteins p100gag-mil and p75gag-raf (of respective relative molecular mass (Mr) 100,000 and 75,000), while the myc oncogene of MH2 is expressed by means of a subgenomic messenger RNA. We have recently demonstrated that p100gag-mil is not a nuclear protein. Here we report that purified p100gag-mil and p75gag-raf exhibit protein kinase activities in vitro which, in contrast to the src-related p130gag-f_s of Fujinami sarcoma virus (FSV) and all other characterized oncogene-encoded protein kinases, phosphorylate serine and threonine but not tyrosine. Both types of protein kinases phosphorylate lipids in vitro.

116. THE COMPLETE CODING SEQUENCE OF THE HUMAN RAF ONCOGENE AND THE CORRESPONDING STRUCTURE OF THE C-RAF-1 GENE

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Nucleic Acids Res; 14(2):1009-15 1986

The complete 648 amino acid sequence of the human raf oncogene was deduced from the 2977 nucleotide sequence of a fetal liver cDNA. The cDNA has been used to obtain clones which extend the human c-raf-1 locus by an additional 18.9 kb at the 5' end and contain all the remaining coding exons.

117. A C-RAF PROTEIN ASSOCIATED WITH KINASE ACTIVITY OBSERVED IN 3T3 CELLS TRANSFORMED BY ACTIVATION OF C-RAF (MEETING ABSTRACT)

Schultz AM, Copeland TD, Mueller R, Rapp U, Oroszlan S
LBI-Basic Res. Program, NCI-Frederick Cancer Res. Facility, Frederick, MD 21701
XXIIth Symposium for Comparative Research on Leukemia and Related Diseases, July 7-11, Hamburg, p. 79, 1985.

We have developed raf-specific antisera (Schultz et al, submitted) with synthetic peptides derived from the sequence of the gag-raf gene of 3611 murine sarcoma virus (MSV), and have used them to identify a 48 KDa protein (p48) in clone F/S1-3-4, which contains an activated c-raf gene (Molders et al, EMBO J, in press). P48 was confirmed to be a product of the c-raf gene by reaction with four different raf-specific antipeptide sera, including one directed to the carboxyl-terminus of c-raf. Unlike the gag-raf proteins of 3611 MSV, p48 is neither glycosylated or myristylated, but like P75gag-raf it is phosphorylated in vivo on serine and threonine residues as determined by high performance liquid chromatography after labeling p48 with 32PO4. Immunoprecipitates of p48 exhibited autophosphorylation but did not phosphorylate immunoglobulin heavy chain. The c-raf gene is capable of encoding a protein substantially larger than p48. In both F/S1-3-4 cells and 3611 MSV, a transforming raf protein thus shows loss of the amino-terminal region of c-raf. (2 Refs)

118. NUCLEOTIDE SEQUENCE OF AVIAN RETROVIRAL ONCOGENE V-MIL: HOMOLOGUE OF MURINE RETROVIRAL ONCOGENE V-RAF

Sutrave P, Bonner TI, Rapp UR, Jansen HW, Patschinsky T, Bister K
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland.
Nature; 309(5963):85-8 1984

Eukaryotic cells contain genes termed proto-oncogenes (c-onc) which have the potential to transform cells in culture and induce tumours in vivo. Most of these genes have been

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identified by their occasional incorporation into retroviral genomes which can act as natural transducing vectors for these and perhaps other cellular genes. Cell-derived oncogenes of retroviruses (v-onc) are associated mostly with the induction of mesenchymal tumours whereas carcinoma induction is rare. One of these rare carcinoma-inducing viruses is the acutely transforming avian retrovirus MH2 (refs 3-5). Recently we and others have shown that this virus carries a novel putative oncogene, v-mil, in addition to the known oncogene v-myc. While the transforming ability of v-mil has not been directly established, we have recently discovered by hybridization analysis that v-mil is homologous to v-raf (ref. 9), the transforming gene of the murine retrovirus 3611 MSV (ref. 10). Both viruses express the mil /raf oncogene product as a gag-fusion polyprotein, while the myc oncogene of MH2 is expressed via a subgenomic mRNA. Here we report the complete nucleotide sequence of v-mil and compare it with that of v-raf. The 80% homology between the nucleotide sequences and the 94% homology between the predicted amino acid sequences of the two viral genes clearly indicate that these are the avian and murine forms of the same gene. Comparison of the two sequences with that of the human cellular homologue (T. I. Bonner et al., manuscript in preparation) indicates that v-raf has more 3' untranslated sequences while v-mil has additional sequences from two 5' exons of the cellular homologue. Although the mil /raf amino acid sequences reveal partial homology to that of the v-src product, no tyrosine-specific protein kinase activity is detected for the gag-mil and the gag-raf hybrid proteins.

119. THE HUMAN AND MOUSE CELLULAR HOMOLOGUES OF THE RAF/MIL ONCOGENE (MEETING ABSTRACT)

Rapp UR. Bonner T. Goldsborough M. Sutrave P. Oppermann H. Seeburg P
Lab. of Viral Carcinogenesis, NCI, Frederick, MD
XV Meeting of the European Tumour Virus Group. September 19-24, 1984. Urbino, Italy. p. 24, 1984.

We have identified two loci in the human genome which are related to the raf/mil oncogene. One of these, c-raf-2, is a processed pseudogene. The active gene, c-raf-1, contains 11 exons which are homologous to v-mil, 9 of which are also homologous to v-raf. These exons span more than 21 kb and account for 1.26 kb of coding sequence and 0.96 kb of 3' untranslated sequence, the 3' end of the message having been defined by cDNA cloning. Since the message in humans is 3.5 kb, there is another 1.3 kb from the 5' end of the message which we are currently characterizing using long cDNA clones. Comparison with the mouse cellular homologue reveals extensive homology in the 2 kb region immediately 3' of the poly(A) site. The function of this conserved region is unclear but it might represent a transcription termination site located substantially 3' of the poly(A) site. A mechanism of transduction is suggested by the sequences of c-raf-1 at the points corresponding to the helper-virus-oncogene junctions of v-raf. At the 5' junction there is a 20 nucleotide sequence which is identical in c-raf-1 and v-raf and which matches 16 of 19 corresponding bases of Moloney p30 sequence. At the 3' junction there is a stretch of 6 nucleotides which are identical in c-raf-1, v-raf and MoLV polymerase. Taken together these homologies indicate that the raf gene was transduced by homologous recombination between helper proviral DNA and the raf cellular homologue. The biological activity of c-raf-1 has been demonstrated by transfection using DNA constructs in which portions of v-raf have been replaced by the corresponding c-raf-1 sequence. Thus, point mutations are not required to activate the gene. However, it is possible that the gene is activated by removal of its 5' end. Expression of c-raf-1 proteins in human tumor and normal tissues as well as the biological activity of expression-vector produced c-raf-1 protein was presented.

120. EXPRESSION OF THE V-MIL ENCODED P100GAG-MIL IS REQUIRED FOR TRANSFORMING AND MITOGENIC PROPERTIES OF MH2 IN CHICK EMBRYO NEURORETINAL CELLS (MEETING ABSTRACT)

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XV Meeting of the European Tumour Virus Group. September 19-24, 1984. Urbino, Italy. p. 25, 1984.

The avian carcinoma virus MH2 contains two unrelated oncogenes: v-mil and v-myc. In MH2 transformed cells, v-mil is expressed as the C-terminal portion of a 100 Kd polyprotein (P100gag-mil). We previously reported that chick embryo neuroretinal (NR) cells, which normally do not multiply in vitro, are transformed and induced to proliferate after infection with MH2. In contrast, NR cells infected with MC29, CMII or OK-10, which carry only v-myc, retain their normal morphology and limited growth capacity. To identify the genetic information of MH2 responsible for transformation and proliferation of NR cells, we first isolated two mutants MH2-Cl 16 and Cl-25 defective in these properties. Both viruses, which efficiently transformed quail embryo fibroblasts (QEF), failed to direct the synthesis of P100gag-mil. QEF transformed by either mutant contained only one species of MH2-specific RNA of 2.8 Kb hybridizing only to v-myc. Accordingly, digestion of DNAs from mutant infected cells with enzymes cleaving the proviral DNA only in the LTR, generated a fragment of 2.2 Kb hybridizing to v-myc but not to v-mil, indicating that both MH2 Cl 16 and Cl 25 suffered extensive deletions in the gag-mil region. We also constructed in vitro two mutants of MH2, pOB and pOB K, by deleting most of the gag sequence in the plasmid pMH2-Hd containing a biologically active DNA representation of MH2. Non producer clones of QEF transformed by either mutant did not synthesize P100gag-mil presumably as a consequence of a frameshift mutation affecting synthesis of this protein. Both viruses also failed to establish transformation and proliferation of NR cells. These results show that the transforming and mitogenic properties of MH2 in NR cells are correlated with the expression of P100gag-mil. Whether the product of v-mil is sufficient to alter the phenotype of NR cells or whether these alterations require cooperation between v-mil and v-myc is not yet established. Therefore, construction of MH2 mutants deleted in v-myc and assay of their properties in NR cells are under investigation.

121. ANALYSIS OF THE MIL ONCOGENE OF MH2 AND ITS CELLULAR COUNTERPART (MEETING ABSTRACT)

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INSERM U186, Inst. Pasteur de Lille, Lille, France
XV Meeting of the European Tumour Virus Group. September 19-24, 1984. Urbino, Italy. p. 15, 1984.

The avian carcinoma virus MH2 has been shown to contain a second cell-derived oncogene, v-mil, in addition to v-myc. The v-mil domain is expressed as part of a 100 kD gag-mil fusion protein whereas the v-myc domain is expressed as a 57 or 61/63 kD protein translated from a subgenomic mRNA. The complete nucleotide sequence of the 1.1 kbp v-mil domain has been determined. Several regions of the v-mil gene were found to be homologous to the oncogenes of the src gene family. This homology however, does not include the tyrosine phosphoacceptor site characteristic of many members of the src gene family. We have isolated from a

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chicken genomic library, overlapping phages spanning 16 kbp of cellular DNA and containing the cellular counterpart of v-mil. Restriction enzymes and heteroduplex analyses have shown that v-mil homologous sequences are organized in at least 10 exon-like domains correspon ding to the 3' end of the c-mil locus. Northern blots and hybridization analyses suggest that the entire c-mil locus spans at least 25 kbp of chicken cellular DNA. The c-mil gene is expressed in the chicken as a single RNA species of 4.0 kb. Expression of the 4.0 kb c-mil RNA although particularly prominent in heart, muscle and bone marrow tissues, was detected in several transformed cell-lines as well as normal tissues. These molecular parameters suggest for c-mil a basic role in the economy of the cell, possibly in cell growth, since v-mil has a mitogenic effect on quail neuroretinal cells.

122. PRIMARY STRUCTURE OF V-RAF: RELATEDNESS TO THE SRC FAMILY OF ONCOGENES

Mark GF. Rapp UR
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland.
Science; 224(4646):285-9 1984

A replication-defective, acute transforming retrovirus (murine sarcoma virus 3611) was isolated from mouse and molecularly cloned. The nucleotide sequence of 1.5 kilobases encompassing the transforming gene (v-raf) was determined. This sequence, which predicts the amino acid sequence of a gag-raf fusion protein, terminates 180 nucleotides from the 3' end of the acquired cellular sequence. Comparison of the predicted amino acid sequence of v-raf with the predicted amino acid sequences of other oncogenes reveals significant homologies to the src family of oncogenes. There is a lack of homology within the sequence of the tyrosine acceptor domain described for the phosphotyrosine kinase members of the src family of transforming proteins. Phylogenetic arrangement of this family of oncogenes suggests that tyrosine-specific phosphorylation may be a recently acquired activity.

123. INTEGRATION OF TRANSFECTED LTR SEQUENCES INTO THE C-RAF PROTO-ONCOGENE: ACTIVATION BY PROMOTER INSERTION

Molders H. Defesche J. Muller D. Bonner TI. Rapp UR. Muller R
European Molecular Biology Laboratory, Heidelberg, FRG.
EMBO J; 4(3):693-8 1985

A malignant cell line (clone S1) isolated after co-transfection of normal NIH3T3 DNA and Moloney leukemia virus long terminal repeat (Mo-LTR) sequences has previously been described to contain an activated c-raf oncogene. Here, we report the isolation by molecular cloning and the structural analysis of the LTR-activated c-raf gene. As shown by Southern blot and nucleotide sequence analyses, the transfected Mo-LTR sequences integrated into the 5th intron of the endogenous c-raf proto-oncogene. This intragenic LTR insertion led to the expression of high levels of LTR-U5-c-raf hybrid transcripts indicating an initiation of transcription from the Mo-LTR promoter. Transcriptional activation of c-raf is accompanied by the synthesis of large amounts of cytoplasmic c-raf protein. Immunoblot analysis suggests that the proteins encoded by the LTR-activated c-raf gene are truncated compared with th e normal c-raf gene product(s). Our results indicate a promoter insertion mechanism of c-raf activation.

B.
Cytoplasmic Protein Products of Cellular Oncogenes: Other Activities

1.
ras protein products

124. PURIFICATION AND CHARACTERIZATION OF HUMAN H-RAS PROTEINS EXPRESSED IN ESCHERICHIA COLI

Gross M. Sweet RW. Sathe G. Yokoyama S. Fasano O. Goldfarb M. Wigler M. Rosenberg M
Molecular Genetics Department, Smith Kline and French Laboratories, Philadelphia, Pennsylvania 19101.
Mol Cell Biol; 5(5):1015-24 1985

The full-length normal and T24 mutant human H-ras proteins and two truncated derivatives of the T24 mutant were expressed efficiently in Escherichia coli. The proteins accumulated to 1 to 5% of total cellular protein, and each was specifically recognized by anti-ras monoclonal antibodies. The two full-length proteins as well as a carboxyl-terminal truncated derivative (deleted for 23 amino acid residues) were soluble upon cell lysis and were purified to 90% homogeneity without the use of denaturants. In contrast, an amino-terminal truncated ras derivative (deleted for 22 amino acid residues) required treatment with urea for its solubilization. The guanine nucleotide binding activity of these four proteins was assessed by a combination of ligand binding on proteins blots, immunoprecipitation, and standard filter binding procedures. The full-length proteins showed similar binding kinetics and a stoichiometry approaching 1 mol of GTP bound per mol of protein. The showed similar binding kinetics and a stoichiometry approaching 1 mol of GTP bound per mol of protein. The carboxyl-terminal truncated protein also bound GTP, but to a reduced extent, whereas the amino-terminal truncated protein did not have binding activity. Apparently, the carboxyl-terminal domain of ras, although important for transforming function, does not play a critical role in GTP binding.

125. COMPARATIVE ANALYSIS OF P21 PROTEINS FROM VARIOUS CELL TYPES BY TWO-DIMENSIONAL GEL ELECTROPHORESIS

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J Cell Biochem; 29(3):253-63 1985

The products of the ras gene family are related proteins at a molecular weight of 21 kDa, designated p21. In the present study we used two-dimensional gel electrophoresis to compare p21 proteins from five different normal and malignant cell lines. Using a known protein (3H-labeled translation initiation factor [eIF-4D]) as a standard internal marker for isoelectric point (pI), we show that p21 proteins from various cells differ only slightly in molecular weight (21-24 kDa) but express a wide variety in charge (pI 4.8 to 7) that could only be detected by the use of two-dimensional gel electrophoresis. p21 in NIH/3T3 cells was expressed as a single protein, which migrated at 21 kDa and pI 5.1. This peptide, which is probably the product of the normal cellular ras gene, was also detected in normal human lymphocytes. The synthesis of this peptide was not elevated in the transformed cells. However, transformation of NIH/3T3 fibroblasts and of human leukocytes was found to be associated with expression of qualitatively different forms of p21 peptides. Four additional p21-associated peptides of identical molecular weight (23

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kDa), but multiple charge forms, were detected selectively in Kirsten murine sarcoma virus-transformed NIH/3T3 cells. Transformation of cells with Harvey murine sarcoma virus was found to be associated with prominent expression of two major pairs of p21-associated proteins, one at 21 kDa (pI, 5.2 and 5.3) and the other at 23 kDa (pI, 5.1 and 5.2). In HL-60 leukemic cells there was an additional, more acidic form (pI 5.0) of p21, which appeared to be absent or reduced in normal human lymphocytes. These results indicate that p21 from viral origin or cellular origin might be expressed in the cells in multiple charge forms. The capability to distinguish multiple forms of p21 and slight charge modifications associated with malignancy should call for the use of 2-D gel electrophoresis as an important tool in future studies involving p21 proteins.

126. NO EVIDENCE FOR STRUCTURAL OR FUNCTIONAL IDENTITY BETWEEN P24/CD9 AND P21/RAS

LeBien TW, Kersey JH
Department of Laboratory Medicine/Pathology, University of Minnesota, Minneapolis 55455
Leuk Res; 9(12):1565-70 1985

Experiments were undertaken to test the hypothesis that the p24/CD9 cell surface molecule (originally detected on acute lymphoblastic leukemia cells) and the p21 protein encoded by the ras gene family are related. Immunoprecipitation and guanine nucleotide binding assays conducted with BA-2 and anti-p21/ras monoclonal antibodies showed no evidence that p24/CD9 is structurally or functionally related to p21/ras.

127. BIOCHEMICAL PROPERTIES OF A HIGHLY PURIFIED V-RASH P21 PROTEIN OVERPRODUCED IN ESCHERICHIA COLI AND INHIBITION OF ITS ACTIVITIES BY A MONOCLONAL ANTIBODY

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Mol Cell Biol; 5(6):1449-55 1985

The v-rasH oncogene of Harvey murine sarcoma virus encodes a 21,000-dalton p21 protein which has been expressed at a high level as a fusion protein in Escherichia coli. We have purified the p21 to over 90% in purity without the use of any detergent or protein denaturant. The purified p21 possesses full biochemical activities of GTP/GDP binding, autokinase, and GTPase. Scatchard analysis indicates a single class of binding sites with Kd values of 0.83 X 10(-8)M for GTP and 1.0 X 10(-8)M for GDP. The binding site can be specifically labeled with a [3H]GTP photoaffinity analog, P3-(4-azidoanilido)-5' GTP. To probe for the active center of p21, we used a battery of six monoclonal antibodies to p21 to examine their effects on p21 activities. We found that only one monoclonal antibody, Y13-259, was capable of inhibiting both GTP/GDP binding and autokinase enzymatic activities, suggesting that these p21 activities are related activities conferred by a single active center within the p21 molecule. These observations together with the recent finding that microinjection of the same monoclonal antibody into NIH 3T3 cells specifically blocks p21 in vivo function (Mulcahy et al., Nature [London] 313:241, 1985) strongly suggest that p21 in vitro activities are responsible for its cellular function.

128. HARVEY MURINE SARCOMA VIRUS P21 RAS PROTEIN: BIOLOGICAL AND BIOCHEMICAL SIGNIFICANCE OF THE CYSTEINE NEAREST THE CARBOXY TERMINUS

Willumsen BM, Norris K, Papageorge AG, Hubbert NL, Lowy DR
Finsen Institute, Copenhagen, Denmark
EMBO J; 3(11):2581-5 1984

Previous studies of premature chain termination mutants and in frame deletion mutants of the p21 ras transforming protein encoded by the transforming gene of Harvey murine sarcoma virus (Ha-MuSV) have suggested that the C terminus is required for cellular transformation, lipid binding, and membrane localization. We have now further characterized the post-translational processing of these mutants and have also studied two C-terminal v-rasH point mutants: one encodes serine in place of cysteine-186, the other threonine for valine-187. The Thr-187 mutant was transformation-competent, and its p21 protein was processed normally, as was the p21 encoded by a transformation-competent deletion mutant from which amino acids 166-175 had been deleted. The Ser-186 mutant was defective for transformation. The p21s encoded by the Ser-186 mutant and by the previously described transformation-defective mutants did not undergo the posttranslational processing common to biologically active ras proteins: their electrophoretic migration rate did not change, they remained in the cytosol, and they failed to bind lipid. Since the cell-encoded ras proteins also contain this cysteine, we conclude that this amino acid residue is required for all ras proteins.

129. CHARACTERIZATION OF FUNCTIONAL DOMAINS OF P21 RAS BY USE OF CHIMERIC GENES

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EMBO J; 4(2):407-12 1985

Comparison of the predicted amino acid sequences of different members of the ras family in vertebrates has shown that the N-terminal 120 residues are highly conserved while the C terminus is variable. To test the possible role of the variable residues in cell transformation, chimeras were constructed containing the N-terminal 111 amino acids of the human Ha-ras EJ oncogene and the C terminus of two Drosophila ras genes. We show that one of these constructs which has only 20 conserved residues between positions 121 and 189, can transform rat-1 cells, and the transformed cells are capable of inducing lethal tumors in rats. The second construct containing the C terminus of another Drosophila ras gene exhibits a transforming capacity as well, but only after linkage to a viral transcriptional promoter. These results show that the majority of residues within the C terminus can be replaced without abolishing the transforming potential of p21 ras.

130. STRUCTURAL EFFECTS OF SUBSTITUTIONS ON THE P21 PROTEINS

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Proc Natl Acad Sci USA; 82(11):3596-300 1985

The conformational effects of different amino acid substitutions (lysine, serine, proline, and D-valine) for glycine at position 12 in the p21 oncogene-encoded proteins have been investigated by using conformational energy calculations. The normal cellular gene codes for a glycine at position 12 in the amino acid sequence, in the middle of a hydrophobic p21-(6-15)-decapepetide from Leu-6 to Gly-15. Mutations that cause amino acid substitutions for Gly-12 result in a protein product that produces malignant transformation of cells. We now find that not only are the preferred structures for the lysine- and serine-containing peptides more restricted and more helical than those for the glycine-containing peptide, but the lowest-energy structure for each substituted peptide is exactly the same as that previously found for the peptide with Val-12, suggesting the existence of a 'malignancy-causing' conformation. None of the preferred conformations for the valine-, lysine-, and serine-containing peptides contain chain reversals at positions 11 and 12. However, we find that proline, unlike

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these residues but like glycine, at position 12 causes helix termination at positions 11 and 12, a result that suggests that the p21 protein product with proline at position 12 may exhibit lowered transforming potential, in agreement with the results of recent genetic recombination experiments.

131. CONFORMATIONAL PROPERTIES OF ONCOGENE PRODUCTS (MEETING ABSTRACT)

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Molecular Basis of Cancer: An Interdisciplinary Discussion on Basic and Applied Aspects of Cancer, May 30-June 2, 1982, Buffalo, New York, Roswell Park Memorial Institute, p. 18, 1984.

Sequence alignment of proteins whose structure or function is unknown with proteins with known structure/function relationships presents an avenue to begin to speculate on hitherto unknown conformational or functional properties. The neoplastic transforming activity of the ras gene products has been attributed to small, specific changes in their peptide sequences clustering around positions 12 and 13 and for amino acids 59 to 63. Sequence comparisons of these proteins with mononucleotide binding proteins suggest that the domain around the 12th position exhibits a beta-alpha-beta fold. Conformational calculations on the other hand suggest that the intrinsic nature of the beta-alpha part is alpha-alpha. Here we reexamine the possible structural properties of the domain around the 12th and 13th position by including other oncogene products in the sequence analysis and other proteins whose structure or function is known. Sequence alignment of this region with cro and CI repressors indicate that the region can exhibit alpha alpha properties as evidenced from the conformational calculations and suggest that the oncogene products may exhibit binding properties with double stranded nucleic acids. Sequence tracts found within oncogene products are further compared with tracts in known proteins to elucidate possible regions for oncogene products' proteolytic cleavage and activation. Antigenic determinants are also calculated for several oncogene products and for several structurally known proteins. Comparison of determinants are used to test antigenic relationships between transforming gene products. Results from this analysis show, for example, that P90 gag-yes, the transforming protein of a representative of Class III avian sarcoma viruses, is antigenically related to pp60 v-src. In addition, the calculation of determinants, which are shown to represent surface structural features, suggests that the folding of adenylate kinase and the ras oncogene product p21 may fold in similar ways despite their differences in sequence and the distribution of disulfide bonds.

132. POSTTRANSLATIONAL PROCESSING OF P21 RAS PROTEINS INVOLVES PALMITYLATION OF THE C-TERMINAL TETRAPEPTIDE CONTAINING CYSTEINE-186

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J Virol; 56(2):607-12 1985

The p21 proteins of ras oncogenes are synthesized as precursors in the cytosol. After processing, which involves acylation, the products are associated with the plasma membrane in eucaryotic cells. The p21 overproduced in Escherichia coli, however, is not processed by acylation. A synthetic tetrapeptide of the p21 C terminus is used to identify the acylation site in eucaryotic p21 as cysteine-186. The same peptide of bacterial p21 is not acylated. Although p21 of Harvey murine sarcoma virus-transformed NRK cells can be metabolically labeled with either [3H]palmitate or [3H]myristate, the lipid moiety of the hydrophobic peptide is identified as palmitic acid. We suggest that the enzymatic mechanism for p21 palmitylation may be different from N-terminal myristylation of many other membrane proteins.

133. MUTATION AFFECTING THE 12TH AMINO ACID OF THE C-HA-RAS ONCOGENE PRODUCT OCCURS INFREQUENTLY IN HUMAN CANCER

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Science; 220(4602):1175-7 1983

A point mutation alters the 12th amino acid of the c-Ha-ras oncogene product p21 in a human bladder cancer cell line. This is, at present, the only mutation known to result in a human transforming gene. This mutation may therefore represent a possible target for mutagenesis leading to carcinogenesis in humans. By means of restriction enzyme analysis, 29 human cancers, including 20 primary tumor tissues, derived from organs commonly exposed to environmental carcinogens, were tested for the presence of this mutation. None of ten primary bladder carcinomas exhibited the mutation; nor did nine colon carcinomas or ten carcinomas of the lung. Thus the point mutation affecting the 12th amino acid of the c-Ha-ras gene product, while a valuable model for carcinogenesis, does not appear to play a role in the development of most human epithelial cancers of the bladder, colon, or lung.

134. TRANSIENT REVERSION OF RAS ONCOGENE-INDUCED CELL TRANSFORMATION BY ANTIBODIES SPECIFIC FOR AMINO ACID 12 OF RAS PROTEIN

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Nature; 314(6012):639-42 1985

The proteins encoded by the ras oncogene are thought to trigger expression of the transformed phenotype in some types of cancer cells. In human cells, the ras protein family consists of several members including normal (proto-oncogene) and mutant (oncogene) forms. In general, the proto-oncogene forms are thought to be involved in the normal growth control of cells, while the mutant forms (which apparently result from somatic mutation of the normal ras genes) appear to be responsible, in part, for the loss of normal growth control. On microinjection into living normal cells, the purified ras oncogene protein (p21) induces a characteristic loss of growth control in cells within several hours. The mutant forms of the different ras proteins typically contain a single amino-acid change, usually at position 12 or less frequently at position 61. Here we report that microinjection of antibodies specific for amino acid 12 of the oncogenic v-Ki-ras protein into cells transformed by this protein causes a transient reversion of the cells to a normal phenotype. The fact that this antibody inhibits binding of GTP to the v-Ki-ras protein supports the notion that GTP binding is essential to the transforming function of this oncogene product.

135. BIOCHEMICAL CORRELATES OF PHENOTYPIC REVERSION IN INTERFERON-TREATED MOUSE CELLS TRANSFORMED BY A HUMAN ONCOGENE

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Biochem Biophys Res Commun; 119(1):21-8 1984

Mouse interferon (IFN) induced a phenotypic reversion in RS 485, a clonal line of NIH 3T3 oncogenically transformed

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by a human c-Ha-ras1 gene activated by Ha-MuSV long terminal repeats (LTRs). Transfected c-Ha-ras DNA, unchanged in quantity and distribution, as compared to the parental RS 485 transformed cells, was still present in these revertants; however, there was a significant reduction in the amount of c-Ha-ras specific mRNA and of c-Ha-ras specified p21 protein.

136. THE P21 RAS C-TERMINUS IS REQUIRED FOR TRANSFORMATION AND MEMBRANE ASSOCIATION

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Nature; 310(5978):583-6 1984

The Harvey murine sarcoma virus (Ha-MuSV) transforming gene, v-rasH, encodes a 21,000 molecular weight protein (p21) that is closely related to the p21 proteins encoded by the cellular transforming genes of the ras gene family. The primary translation product (prop21), which is found in the cytosol, undergoes posttranslational modification and the mature protein subsequently becomes associated with the inner surface of the plasma membrane and binds lipid tightly. The p21 proteins have the capacity to bind guanine nucleotides non-covalently in vitro. To assess the biological relevance of these biochemical features of the protein, we have now studied a series of deletion mutants located at or near the C-terminus of the viral p21 protein. Our tissue culture studies indicate that amino acids located at or near the C-terminus are required for cellular transformation, membrane association and lipid binding.

137. MICROINJECTED RAS FAMILY ONCOGENES STIMULATE DNA SYNTHESIS IN QUIESCENT MAMMALIAN CELLS

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Virology; 141(2):333-6 1985

Oncogenes of the ras family stimulate DNA synthesis when microinjected into quiescent mouse and hamster fibroblasts, as detected by in situ autoradiography. The molecularly cloned genomes of Harvey and Kirsten sarcoma viruses, the cloned Harvey ras gene, and the product of the v-ras gene, the p21v-rasH protein, stimulate DNA synthesis in quiescent cells. This stimulation is comparable to the stimulatory activity of the microinjected SV40 T-antigen-coding gene. The demonstration that these oncogenes can stimulate transient DNA synthesis in quiescent cells is relevant to understanding the mechanism by which these genes are able to transform cells in vitro and induce tumors in animals.

138. TRANSFORMATION OF NIH 3T3 CELLS BY MICROINJECTION OF HA-RAS P21 PROTEIN

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Nature; 310(5977):508-11 1984

Alteration in gene structure has been shown to occur in some human tumours. These altered genes, termed oncogenes, were originally identified by their ability to induce foci of transformed cells on transfected mouse 3T3 cultures. The oncogene identified in the EJ/T24 human bladder carcinoma is similar to the transforming gene of BALB and Harvey murine sarcoma virus (MSV) and differs from its counterpart in normal cells by a single amino acid. All three of these Ha-ras genes direct the production of similar proteins (p21). While the ras gene appears to be involved in tumour formation in some situations, its role is unclear. The ras protein product (p21) binds guanine nucleotides and has a unique autophosphorylating activity, but no other enzymatic activity has been found. We report here the injection of purified Ha-ras p21 protein, made in Escherichia coli from the gene of BALB-MSV, into NIH 3T3 cells and show that the purified protein itself is sufficient to induce a transformed morphology. In addition, the injected protein stimulates quiescent cells to enter the S-phase of the cell cycle. This result clearly demonstrates that the ras gene functions directly through the protein product. It also establishes an assay for the protein which depends on its activity within a living cell. The transforming activity of a p21 ras protein equivalent to the product of the normal cellular ras gene, is also demonstrated.

139. P21RAS. HETEROGENEOUS LOCALIZATION IN TRANSFORMED CELLS

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Exp Cell Res; 159(2):441-50 1985

The cellular targets for the Kirsten murine sarcoma virus (KiMSV)-transforming protein, p21ras, are unknown. Other studies have indicated that the mature form of p21 is distributed diffusely on the cytoplasmic face of the plasma membrane. However, after fixation without buffer washes, indirect immunofluorescent staining of sparse cultures revealed a particularly well preserved cellular architecture and a strikingly heterogeneous subcellular distribution of p21 in transformed normal rat kidney (NRK) cells but not in their untransformed counterparts. The transformed cells included A KiMSV-transformed NRK line. NRK cells newly transformed with KiMSV. A temperature-sensitive (ts) KiMSV-transformed NRK line. An uninfected, spontaneously transformed NRK line in which p21 was neither phosphorylated nor overproduced. In the tsKNRK line p21 was abundant at both permissive and non-permissive temperatures; however, its distribution was heterogeneous at the permissive temperature only. Observation of this array of cells indicates that the transformation-associated p21 distribution does not require overexpression of the gene, nor phosphorylation of the protein, nor the viral oncogene. Furthermore, it is reversible in the tsKNRK cells, and so appears to be highly correlated with acquisition of a transformed morphology. Accumulations of p21 occurred preferentially in subcellular locations similar to those where ruffles were observed by phase contrast microscopy and lamellar and villous extensions were observed by scanning electron microscopy (SEM). Since enhanced ruffling is a morphological correlate of transformation in a variety of cells, the distribution of p21 observed here may relate to its function as a transforming molecule.

140. REQUIREMENT FOR C-RAS PROTEINS DURING VIRAL ONCOGENE TRANSFORMATION

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Nature; 320(6062):540-3 1986

Many retroviral oncogenes have been classified into one of several categories based on structure, enzymology and cellular localization. These genes originated from host cells and are probably derived from genes normally involved in the control of cell proliferation. The cellular counterparts of three oncogenes have been identified as a growth factor or growth factor receptor; related oncogenes include receptor-like membrane proteins which often express tyrosine kinase activity. These growth factor-related oncogenes are structurally and biochemically distinct from the membrane-associated ras gene family, which bind and hydrolyse GTP. Oncogenes localized primarily in the cytoplasm which probably have

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serine kinase activity, have also been identified. Although the structure and biochemistry of many oncogenes have been extensively studied, relatively little is known about the functional relationships of oncogene proteins within the cell. An opportunity to study such interaction is provided by the identification of a monoclonal antibody that neutralizes cellular ras proteins when microinjected into cells. It has been shown previously that the injected antibody inhibits the initiation of S-phase in NIH 3T3 cells. In the present study we injected this monoclonal antibody into NIH 3T3 cells transformed by a variety of oncogenes. The results show that transformation by three growth factor receptor-like oncogenes depends on c-ras proteins, while transformation by two cytoplasmic oncogenes appears to be independent of c-ras protein.

141. MUTANT RAS-ENCODED PROTEINS WITH ALTERED NUCLEOTIDE BINDING EXERT DOMINANT BIOLOGICAL EFFECTS

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Proc Natl Acad Sci USA; 83(4):952-6 1986

We report that residues Lys-16 and Asp-119 play critical roles in the guanine nucleotide binding and, consequently, the biological function of the Ha-ras-encoded protein (Ha). Substitution of an asparagine residue for Lys-16 reduces the affinity of Ha for GDP and GTP by a factor of 100 but does not alter the specificity of nucleotide binding. The replacement of Asp-119 with an alanine residue reduces the affinity of Ha for GDP and GTP by a factor of 20 and reduces the relative affinity of Ha for GDP over IDP from 200-500 to 10. Based on these observations, a structural model for the GDP/GTP-binding site of Ha is proposed. By microinjecting purified proteins into NIH 3T3 cells, we observed that the ability of [Ala119]Ha to induce changes characteristic of cellular transformation was much greater than that of normal Ha and similar to that of the oncogenic [Val12, Thr59]Ha. In this assay, [Asn16]Ha and [Val12, Asn16, Thr59]Ha were similar in potency to normal Ha. In yeast cells, Ha proteins with reduced nucleotide affinity exert a dominant temperature-dependent lethality that is avoided by the coexpression of the activated yeast ras gene [Ala18, Val19]RAS2. We interpret the biological consequences of reducing the nucleotide affinity of ras proteins in terms of two opposing factors: a growth-promoting effect, resulting from an increase in the GDP-GTP exchange rate, and a growth-limiting effect, resulting from an increase in the nucleotide-free ras protein species.

142. TWO CLASSES OF CAMP ANALOGS SYNERGISTICALLY INHIBIT P21 RAS PROTEIN SYNTHESIS AND PHENOTYPIC TRANSFORMATION OF NIH/3T3 CELLS TRANSFECTED WITH HA-MUSV DNA

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Biochem Biophys Res Commun; 130(3):1193-200 1985

Factors that control cellular proliferation might do so by regulating quantitative expression of viral or cellular oncogenes. Since the growth regulatory effect of cAMP is well-known, the effect of cAMP on ras gene expression was examined on Ha-MuSV-transformed 13-3B-4 cells (NIH-3T3) grown in chemically defined serum-free medium. Treatment of cells with two classes of cAMP analogs which are selective for the two different cAMP-binding sites of type II protein kinase, in combination, synergistically inhibited both p21 ras protein synthesis and phenotypic transformation. The inhibition was also demonstrated with these analogs singly but at higher concentrations. The decrease in p21 synthesis was inversely correlated with an increase in the RII cAMP receptor protein, the regulatory subunit of type II protein kinase. These results suggest a role for cAMP and its receptor protein in the regulation of v-rasH oncogene expression.

143. THE RAS ONCOGENE PRODUCT P21 IS NOT A REGULATORY COMPONENT OF ADENYLATE CYCLASE

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Nature; 317(6032):71-2 1985

Harvey (Ha-MSV) and Kirsten (Ki-MSV) murine sarcoma viruses induce tumours in animals and transform various cells in culture because of the expression of the ras oncogene product, p21 (ref. 1). Proto-oncogenes homologous with these genes are highly conserved evolutionarily and activated ras oncogenes have been detected in many human cancers. Whether c-ras oncogenes are directly responsible for human carcinogenesis is uncertain; however, it is clear that p21 mediates virus-induced transformation, although by an unknown mechanism. Epithelial and fibroblast cell lines transformed with Ha-MSV and Ki-MSV express p21 (ref. 8) and exhibit reduced adenylate cyclase activity. Like the guanine nucleotide regulatory proteins, Ns and Ni, which mediate stimulation and inhibition, respectively, of adenylate cyclase, p21 is a membrane-associated GTP binding protein, which exhibits GTPase activity. These similarities suggest that p21 and the adenylate cyclase regulatory proteins are related in cellular function, and that p21 depresses adenylate cyclase by inhibiting the activity of Ns or acting as Ni. We have therefore now examined the structural and functional similarities between p21 and Ns and Ni and find no evidence that p21 regulates adenylate cyclase activity by acting as one of these regulatory proteins.

144. ATP-BINDING SITE OF ADENYLATE KINASE: MECHANISTIC IMPLICATIONS OF ITS HOMOLOGY WITH RAS-ENCODED P21, F1-ATPASE, AND OTHER NUCLEOTIDE-BINDING PROTEINS

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Proc Natl Acad Sci USA; 83(4):907-11 1986

The MgATP binding site of adenylate kinase, located by a combination of NMR and x-ray diffraction, is near three protein segments, five to seven amino acids in length, that are homologous in sequence to segments found in other nucleotide-binding phosphotransferases, such as myosin and F1-ATPase, ras p21 and transducin GTPases, and cAMP-dependent and src protein kinases, suggesting equivalent mechanistic roles of these segments in all of these proteins. Segment 1 is a glycine-rich flexible loop that, on adenylate kinase, may control access to the ATP-binding site by changing its conformation. Segment 2 is an alpha-helix containing two hydrophobic residues that interact with the adenine-ribose moiety of ATP, and a lysine that may bind to the beta- and gamma-phosphates of ATP. Segment 3 is a hydrophobic strand of parallel beta-pleated sheet, terminated by a carboxylate, that flanks the triphosphate binding site. The various reported mutations of ras p21 that convert it to a transforming agent all appear to involve segment 1, and such substitutions may alter the properties of p21 by hindering a conformational change at this segment. In F1-ATPase, the flexible loop may, by its position, control both the accessibility and the ATP/ADP equilibrium constant on the enzyme.

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145. DECREASED HORMONE-STIMULATED ADENYLATE CYCLASE ACTIVITY IN NIH3T3 CELLS EXPRESSING THE EJ HUMAN BLADDER CARCINOMA ONCOGENE (EJ-RAS) (MEETING ABSTRACT)

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Proc Annu Meet Am Assoc Cancer Res; 27:427 1986

Recent studies have shown that the 21 kilodalton protein (p21) Harvey-ras gene product shares sequen ce homology with mammalian guanine nucleotide binding proteins (G-proteins, Hurley et al, Science 226, p. 860). Since one function of G-proteins in vertebrate cells is the regulation of adenylate cyclase (AC) responsiveness, these data suggested that p21 may also regulate AC in cells. We determined both in intact NIH3T3 cells transformed by EJ-ras DNA and in membranes isolated from these cells that the unstimulated and hormone-stimulated AC activity is significantly decreased compared with control cells; thus, the levels of cyclic AMP which accumulate in EJ-ras transformed cells (determined by radioimmunoassay) were down 42% in unstimulated cells and down 78% and 93% in prostaglandin-(2.8 uM) or isoproterenol-stimulated (12 uM) cells, respectively, compared to controls. Neither treatment of the EJ-ras transformed cells with pertussis toxin (which ADP-ribosylates Gi) or cholera toxin (which ADP-ribosylates Gs) corrected the alterations in AC regulation. Cells transfected with the normal human Harvey-ras gene DNA had intermediate levels of AC hormone-sensitivity; these levels of AC activity were greater than those in EJ-ras transformed cells but less than in control cells. Hormone-stimulated AC activity in cells transfected with the v-src gene DNA were indistinguishable from those in control cells. These data support the hypothesis that both the normal and mutated Harvey-ras p21s are functionally related to mammalian G-proteins. (1 Ref)

146. CYCLIC AMP SUPPRESSES EXPRESSION OF V-RASH ONCOGENE LINKED TO THE MOUSE MAMMARY TUMOR VIRUS PROMOTER

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Biochem Biophys Res Commun; 134(1):430-42 1986

Clone 433.3 of NIH 3T3 cells is a stable carrier of the MMTV LTR:v-rasH chimeric DNA. Only in the presence of dexamethasone (a synthetic glucocorticoid), 433.3 cells exhibit an induced level of p21 transforming protein and phenotypic transformation. N6,O2'-dibutyryl cAMP (DBcAMP) antagonized the effect of dexamethasone in a time - and concentration - dependent manner. DBcAMP (5 X 10(-4)M) added 18 hr prior to the addition of dexamethasone (10(-7)M) almost completely blocked the hormone effect: cells contained levels of p21 20% of that in the cells treated with dexamethasone alone, and formed flat, contact inhibited monolayers. On the basis of these results together with our previous data on mammary carcinomas in vivo, we postulate that cAMP may be an intracellular suppressor acting at a regulatory locus of both cellular and viral ras genes.

147. SUPPRESSION OF VIRAL RAS ONCOGENE EXPRESSION IN NIH 3T3 CELLS BY CYCLIC AMP (MEETING ABSTRACT)

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Proc Annu Meet Am Assoc Cancer Res; 26:61 1985

The rate of proliferation of several mammalian cell lines is inversely related to intracellular leve ls of cyclic AMP (cAMP). Our previous studies support the hypothesis that a key event leading to uncontrolled growth of neoplastic cells may be a reduced responsiveness to the growth regulatory effects of cAMP. In this report we now show that suppression of ras gene expression follows cAMP treatment. Clone 13-3B-4 of NIH 3T3 cells, which had been transfected with Harvey murine sarcoma virus DNA, was used in the present study. When 13-3B-4 cells were grown in the serum free medium containing transferrin and insulin, the cells exhibited phenotypic transformation within 2-3 days of culture: they became round, refractile and piled up, and eventually floated away from the substratum. However, phenotypic transformation of 13-3B-4 cells was completely blocked when the cells were grown in serum free medium containing dibutyryl cyclic AMP (DBcAMP) (5 x 10(-4)M): the cells formed flat, contact inhibited monolayers. Other cAMP analogues, as well as cholera toxin, all inhibited phenotypic transformation in this system. This DBcAMP effect on cell morphology was reversible and was completely blocked when the cells were grown in serum free medium containing epidermal growth factor (20 ug/l). The inhibitory effect of DBcAMP on the cell transformation correlated directly with a decrease in the levels of the 21,000-dalton transforming protein (p21) of the v-rasH gene as revealed by Western blotting analysis of p21. Moreover, the decrease in p21 levels following DBcAMP treatment was inversely correlated with an increase in the levels of the 56K M.W. RII cAMP receptor protein in the cells. These results suggest that cAMP may be an intracellular regulator of ras oncogene.

148. [REGULATION OF CAMP CASCADE BY YEAST RAS GENES]

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Gan To Kagaku Ryoho; 13(3 Pt 2):849-53 1986

In the yeast, Saccharomyces cerevisiae, cell proliferation is regulated by cAMP through cAMP-dependent protein kinase. Cyclic AMP-dependent phosphorylation is involved in the G1 phase of the cell cycle, conjugation, and the post-meiotic stage of sporulation, but inhibition of cAMP-dependent protein phosphorylation is required in order to induce meiotic division. The yeast RAS gene, which is homologous to the H-ras gene, is one of several genes which are related to cAMP-cascade, and its product seems to be a regulatory protein of adenylate cyclase.

149. RAS PROTEINS FUNCTION EXCLUSIVELY TO MODULATE ADENYLATE CYCLASE ACTIVITY IN THE YEAST SACCHAROMYCES (MEETING ABSTRACT)

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DNA; 4(1):64 1985

There are two genes in the yeast Saccharomyces cerevisiae, RAS1 and RAS2, that share extensive homology with each other and with the human proto-oncogene family c-ras. We have evaluated phenotypes associated with various lesions in these genes, examining both strains with reduced RAS expression and strains that have 'activated' RAS expression. Strains in which both RAS genes are inactivated are nonviable, apparently due to a failure to execute entry into the cell cycle. In addition, diploid strains with reduced RAS activity sporulate even in the presence of excess nutrients, a condition that normally inhibits sporulation of wild type strains. Strains in which either RAS gene is intact, or even a strain in which both RAS genes are inactive but which carries a human c-ras gene fused to a yeast promoter, display essentially normal growth properties. Strains carrying a mutant RAS allele (RAS-val19), analogous to a transformation-activating allele of a human c-ras gene, are viable. How-

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ever, such strains exhibit a number of aberrant phenotypes: they display a dominant deficiency in sporulation, they fail to arrest uniformly at the beginning of the cell cycle in response to nutrient starvation, they show a substantial reduction in viability upon nutrient starvation, and they fail to accumulate nutrient reserves, such as glycogen or trehalose. These results are consistent with the hypothesis that strains lacking RAS activity cannot enter the cell cycle, whereas strains expressing an activated RAS gene are constitutive for entry into the cell cycle. Phenotypes of strains with mutant RAS alleles are strikingly similar to those of strains with lesions in the cAMP effector pathway. That is, strains lacking adenylate cyclase (cyrl-) are unable to enter the cell cycle. On the other hand, strains carrying the bcyl allele have the same phenotypic properties as those carrying the RAS-val19 allele bcyl is a mutation that suppresses the lethality in adenylate cyclase deficient yeast by rendering cAMP-dependent protein kinase activity constitutive (Matsumoto et al PNAS 79 2355 (1982)). We have found that bcyl suppresses lethality in ras- strains. In addition, RAS-val19 does not suppress cyrl. Therefore, we conclude that the RAS gene products regulate growth in yeast solely by modulating adenylate cyclase activity. Consistent with this interpretation, we find that intracellular cAMP levels are significantly elevated in RAS-val19 strains and virtually undetectable ras- strains. In addition, membranes from ras-yeast lack the GTP stimulated adenylate cyclase activity present in membranes from wild type cells, and membranes from RAS-val19 strains have elevated levels of an apparently GTP-independent adenylate cyclase activity. Mixing membranes from ras-yeast with membranes from adenylate cyclase deficient yeast reconstitutes a GTP dependent adenylate cyclase. (1 Ref)

150. RAS P21 PROTEINS WITH HIGH OR LOW GTPASE ACTIVITY CAN EFFICIENTLY TRANSFORM NIH/3T3 CELLS

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Cell: 44(4):609-17 1986

We sought to determine whether decreased in vitro GTPase activity is uniformly associated with ras p21 mutants possessing efficient transforming properties. Normal H-ras p21-[Gly12-Ala59] as well as an H-ras p21-[Gly12-Thr59] mutant exhibited in vitro GTPase activities at least fivefold higher than either H-ras p21-[Lys12-Ala59] or H-ras p21-[Arg12-Thr59] mutants. Microinjection of as much as 6 X 10(6) molecules/cell of bacterially expressed normal H-ras p21 induced no detectable alterations of NIH/3T3 cells. In contrast, inoculation of 4-5 X 10(5) molecules/cell of each p21 mutant induced morphologic alterations and stimulated DNA synthesis. Moreover, the transforming activity of each mutant expressed in a eukaryotic vector was similar and at least 100-fold greater than that of the normal H-ras gene. These findings establish that activation of efficient transforming properties by ras p21 proteins can occur by mechanisms not involving reduced in vitro GTPase activity.

151. ACTIVATION OF RAS GENES IN HUMAN TUMORS DOES NOT AFFECT LOCALIZATION, MODIFICATION, OR NUCLEOTIDE BINDING PROPERTIES OF P21

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Cell: 37(1):151-8 1984

A comparison of proteins encoded by normal human ras genes and by mutant rasH or rasK genes activated in human carcinomas revealed no changes in subcellular localization, posttranslational modification, or guanine nucleotide binding associated with activation. Subcellular fractionation indicated that both normal and activated ras proteins were associated exclusively with the membrane fraction. Furthermore, both normal and activated ras proteins exhibited similar degrees of posttranslational acylation. The KD for dGTP binding was 1.0-2.2 X 10(-8) M, with no consistent differences between normal and activated ras proteins. In addition, a survey of 13 possible competing nucleotides revealed no differences in the specificity of nucleotide binding associated with ras gene activation. These results indicate that structural mutations which activate ras gene transforming activity do not alter the protein's known biochemical parameters and in particular do not affect the protein's intrinsic ability to bind guanine nucleotides.

152. EPIDERMAL GROWTH FACTOR STIMULATES GUANINE NUCLEOTIDE BINDING ACTIVITY AND PHOSPHORYLATION OF RAS ONCOGENE PROTEINS

Kamata T. Feramisco JR
Cold Spring Harbor Laboratory. New York.
Nature: 310(5973):147-50 1984

Several human tumour cell lines contain genes that can transform NIH 3T3 cells into malignant cells. Certain genes have been classified as members of the ras oncogene family, namely, Ha-ras, Ki-ras or N-ras. The proteins encoded by the ras family are generally small (Ha-ras, for example, encodes a protein of molecular weight 21,000 named p21), and are associated with the inner surface of the plasm a membrane. The only known biochemical property common to all forms of the ras proteins is the ability to bind guanine nucleotides, a property which may be closely related to the transforming ability of ras proteins. A GTP-dependent, apparent autophosphorylation (on threonine 59) activity has been identified only in the case of the v-Ha-ras protein. Although the role of these biochemical activities in the transformation process remains unclear, we have initiated studies to determine the possible biochemical interactions of ras proteins with other membrane components. We report here the evidence that epidermal growth factor enhances the guanine nucleotide binding activity of activated c-Ha-ras or v-Ha-ras p21, and phosphorylation of v-Ha-ras p21, suggesting that some mitogenic growth factors may regulate those activities.

153. ANTIBODIES SPECIFIC FOR AMINO ACID 12 OF THE RAS ONCOGENE PRODUCT INHIBIT GTP BINDING

Clark R. Wong G. Arnheim N. Nitecki D. McCormick F
Department of Human Genetics. Cetus Corporation. Emeryville. CA 94608.
Proc Natl Acad Sci USA: 82(16):5280-4 1985

An antibody (anti-p21ser) was raised against a ras p21-related synthetic peptide and was able to recognize specifically the substitution of serine for glycine at amino acid 12 of p21. This substitution causes oncogenic activation of p21. Anti-p21ser was found to immunoprecipitate v-Ki-ras p21 and to strongly inhibit its ability to autophosphorylate and to bind GTP in an immunoabsorption assay. Furthermore, binding of the antibody to p21 was specifically inhibited by GTP or GDP, suggesting that amino acids around position 12 are part of the GTP/GDP binding site. These results, taken together with the observation that the microinjection of anti-p21ser into cells transformed by v-Ki-ras p21 causes a transient reversion of the cells to a normal phenotype [Feramisco, J. R., Clark, R., Wong, G., Arnheim, N., Milley, R. and McCormick, F. (1985) Nature (London) 314, 639-642], support the idea that interaction of p21 with guanine nucleotides is crucial to the transforming function of this protein.

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154. INTRINSIC GTPASE ACTIVITY DISTINGUISHES NORMAL AND ONCOGENIC RAS P21 MOLECULES

Gibbs JB. Sigal IS. Poe M. Scolnick EM
Virus and Cell Biology Research, Merck Sharp and Dohme Research Laboratories, West Point, PA.
Proc Natl Acad Sci USA; 81(18):5704-8 1984

The 21-kilodalton protein (p21) encoded by normal cellular Harvey-ras has been expressed in Escherichia coli as a fusion protein by using the pUC8 vector and has been purified to greater than 95% homogeneity by ion-exchange chromatography and gel filtration. The purified protein molecules possess intrinsic GTPase activity on the basis of the following criteria: (i) elution of the GTPase activity with p21 GDP-binding activity in two different chromatography systems, (ii) parallel thermal inactiva tion of GTPase activity and p21 GTP-binding activity, and (iii) immunoprecipitation of the GTPase activity with monoclonal antibodies to p21. At 37 degrees C, the rate of GTP hydrolysis by the purified normal p21 assayed in solution was 5.3-6.6 mmol/min per mol of p21. The rate of GTP hydrolysis by a form of p21 [Val12] encoded by a human oncogene was significantly lower (1.4-1.9 mmol/min per mol of p21). The presence of a threonine phosphate acceptor site at residue 59 also decreased p21 GTPase activity. For regulatory proteins that use GTP as part of their biochemical mechanism, the hydrolysis of GTP to GDP reverses the biological activity of the respective proteins. The observation that oncogenic forms of p21 lose GTPase activity suggests that GTP hydrolysis may be a biochemical event that inactivates the growth-promoting effects of a p21 X GTP complex.

155. HPLC ANALYSIS OF GUANINE NUCLEOTIDE CONTENT OF PURIFIED VIRAL HARVEY RAS P21 EXPRESSED IN ESCHERICHIA COLI (MEETING ABSTRACT)

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Merck Sharp and Dohme Res. Labs, Rahway, NJ 07065
Fed Proc; 44(4):1209 1985

Harvey murine sarcoma virus transforms NIH 3T3 cells by means of a 21,000 dalton protein p21, which is encoded from the v-ras oncogene. Viral Harvey ras p21 has been expressed in Escherichia coli as a biochemically-active fusion protein [Stein et al, J Virol 50, 343 (1984)]. When this p21 is purified by a combination of ion-exchange (DE-52) and gel-permeation (G75) chromatography, it contains near stoichiometric amounts of GDP, as shown by HPLC analysis on an ion-exchange column. This noncovalently-associated GDP is also seen in the UV absorption spectrum of the purified protein and in the UV spectrum of HC104-precipitated p21. The HPLC analyses were done on a Varian LC-5000 fitted with a 4 x 30 cm AX-5 Micropak column at room temperature. The eluate of the column is observed at 252 nm (8 nm bandpass) on a Varian UV-50 detector. The column is eluted at a flow rate of 1.0 ml/min with a linear gradient from 100% 10 mM H3PO4, adjusted to pH 3.00 with 1M KOH, to 100% 750 mM 0.75M KH2PO4 over 20 min, followed by elution with the latter buffer for 50 min. Both heat-precipitated (100 C for 15 sec) and HC104-precipitated (0.4M for 15 min at 0 C) p21 released one mole of GDP per mole p21. (1 Ref)

156. A PRODUCT OF YEAST RAS2 GENE IS A GUANINE NUCLEOTIDE BINDING PROTEIN

Tamanoi F. Walch M. Kataoka T. Wigler M
Cold Spring Harbor Laboratory, NY.
Proc Natl Acad Sci USA; 81(22):6924-8 1984

Yeast Saccharomyces cerevisiae contains two genes, RAS1 and RAS2, which show remarkable homology to mammalian ras genes. To characterize these gene products, we have expressed the RAS2 gene in yeast using an inducible GAL10 promoter. After labeling with [35S]methionine and immunoprecipitating with a monoclonal antibody Y13-259, which reacts with p21 encoded by mammalian ras genes, a major band hav ing an apparent molecular weight of 41,000 is detected. This band has also been identified in cell-free translation products of polyadenylated RNA extracted from yeast cells grown in the presence of galactose. Crude extracts of cells expressing the RAS2 gene exhibit guanine nucleotide binding activity. This is detected by incubation with [3H]GDP followed by immunoprecipitation with the antibody Y13-259. The binding of labeled GDP is inhibited by a 20-fold excess of GDP, GTP, and, to a lesser extent, by UTP, a characteristic similar to that possessed by the mammalian ras proteins. However, the activity of the yeast protein differs from that of the mammalian proteins in its strong dependence on temperature. The guanine nucleotide binding activity provides an assay to purify the yeast protein.

157. REGIONAL HOMOLOGY IN GTP-BINDING PROTO-ONCOGENE PRODUCTS AND ELONGATION FACTORS

Halliday KR
Life Sciences Division, Los Alamos National Laboratory, New Mexico.
J Cyclic Nucleotide Protein Phosphor Res; 9(6):435-48 1983

The mammalian ras proteins and the bacterial elongation factors share the ability to interact with guanine nucleotides. Comparison of the amino acid sequences has revealed the presence of multiple homologous regions common to all members of the ras and elongation factor families. Two homologous regions share sequence similarities and predicted secondary structure with areas of the elongation factors which have been implicated in GTP binding, suggesting that these regions are part of a common GTP-binding domain. Two other homologous regions contain critical amino acids for the activation of the transforming potential of the mammalian ras proteins. The predicted secondary structure containing residue 61, but not 12, is the same for the ras proteins and elongation factors. It is proposed that the aligned homologous regions surrounding position 12 constitute common functional binding sites with different specificities; by analogy to other GTP-binding proteins, a likely candidate to interact at such a site is a GTPase-subunit. (43 Refs)

158. COMPLETE NUCLEOTIDE SEQUENCES OF THE T24 HUMAN BLADDER CARCINOMA ONCOGENE AND ITS NORMAL HOMOLOGUE

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Nature; 302(5908):33-37 1983

DNA sequence analysis of the activated oncogene from the T24 human bladder carcinoma line and two alleles of its normal cellular progenitor (c-Ha-ras-1) indicates that the genes encompass at least four exons, and that only a single point mutation residing within the first exon distinguishes the coding region of both alleles of the normal gene from their activated counterpart. Both versions of the gene encode a protein which is predicted to differ from the corresponding viral gene product at thre e amino acid residues, one of which was previously shown to represent the major site of phosphorylation of the viral polypeptide. (Author abstract) (56 Refs)

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159. MECHANISM OF ACTIVATION OF A HUMAN ONCOGENE

Tabin CJ, Bradley SM, Bargmann CI, Weinberg RA, Papageorge AG, Scolnick EM, Dhar R, Lowy DR, Chang EH
Whitehead Inst. Biomedical Res., Center for Cancer Res., Massachusetts Inst. Technology, Cambridge, MA, 02139
Nature; 300(5888):143-149 1982

The oncogene of the human EJ bladder carcinoma cell lines arose via alteration of a cellular proto-oncogene. Experiments are presented that localize the genetic lesion that led to activation of the oncogene. The lesion has no effect on levels of expression of the oncogene. Instead, it affects the structure of the oncogene-encoded protein. (Author abstract) (45 Refs)

160. RAS ONCOGENE P21 EXPRESSION IS INCREASED IN PREMALIGNANT LESIONS AND HIGH GRADE BLADDER CARCINOMA

Viola MV, Fromowitz F, Oravez S, Deb S, Schlom J
Department of Medicine, State University of New York, Stony Brook 11794.
J Exp Med; 161(5):1213-8 1985

ras Oncogene p21 antigen is present in the most superficial cells of the normal bladder urothelium, as demonstrated by immunohistochemical staining. The pattern and intensity of p21 staining of cells in epithelial hyperplasia and low grade bladder carcinoma were similar to that seen in the normal urothelium. In contrast, epithelial cells in 'premalignant' (dysplastic) lesions and high grade carcinomas exhibited an intense staining reaction for p21 antigen. ras p21 may be a useful marker for the malignant potential of both premalignant lesions and carcinomas of the bladder.

161. A POINT MUTATION IS RESPONSIBLE FOR THE ACQUISITION OF TRANSFORMING PROPERTIES BY THE T24 HUMAN BLADDER CARCINOMA ONCOGENE

Reddy EP, Reynolds RK, Santos E, Barbacid M
Lab. Cellular and Molecular Biology, NCI, NIH, Bethesda, MD, 20205
Nature; 300(5888):149-152 1982

The genetic change that leads to the activation of the oncogene in T24 human bladder carcinoma cells is shown to be a single point mutation of guanosine into thymidine. This substitution results in the incorporation of valine instead of glycine as the twelfth amino acid residue of the T24 oncogene-encoded p21 protein. Thus, a single amino acid substitution appears to be sufficient to confer transforming properties on the gene product of the T24 human bladder carcinoma oncogene. (Author abstract) (23 Refs)

162. MICROINJECTION OF THE ONCOGENE FORM OF THE HUMAN H-RAS (T-24) PROTEIN RESULTS IN RAPID PROLIFERATION OF QUIESCENT CELLS

Feramisco JR, Gross M, Kamata T, Rosenberg M, Sweet RW
Cold Spring Harbor Laboratory, New York.
Cell; 38(1):109-17 1984

Using an E. coli expression-vector system we have efficiently produced, purified, and characterized the full-length, nonfused, protooncogenic and oncogenic (T-24) forms of the human H-ras gene product . These purified ras proteins have been introduced by microinjection into a variety of somatic cells in an effort to examine their function. Within several hours after injection of the oncogenic form of the human H-ras protein into quiescent cells, we observe dramatic morphological changes followed by transient proliferation of the cells. In contrast, microinjection of the normal, protooncogenic form of the ras protein at the same level appears to have only little effect on the cells. Additional experiments indicate that the effect of the ras protein requires entry into the cells, is temporary, is inhibited by cycloheximide or actinomycin D, and is seen only in established cell lines. This experimental approach demonstrates that the bacterially derived and purified human H-ras proteins retain their ability to function when put back into mammalian cells and furthermore, provides a novel assay for transformation induced in established cells by the human H-ras oncogene protein.

163. NUCLEOTIDE SEQUENCE ANALYSIS OF THE T24 HUMAN BLADDER CARCINOMA ONCOGENE

Reddy EP
Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland.
Science; 220(4601):1061-3 1983

The nucleotide sequence of the T24 human bladder carcinoma oncogene was determined, and the coding and noncoding sequences of the genome were identified. The amino acid sequence of p21, the translational product of the T24 oncogene, was predicted from the nucleotide sequence of the oncogene. Comparison of this sequence with that of the normal cellular homolog showed that a single point mutation in the coding sequences of the T24 oncogene resulted in the acquisition of transforming properties. Other differences between the T24 oncogene and its normal cellular homolog were found in the 5' noncoding and 3' noncoding sequences, but these differences appear to be due to polymorphism and do not play a significant role in the transformation process.

164. CHARACTERIZATION OF A HUMAN TRANSFORMING GENE ISOLATED FROM T24 BLADDER CARCINOMA CELLS

Santos E, Pulciani S, Barbacid M
Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland.
Fed Proc; 43(8):2280-6 1984

A human transforming gene present in T24 bladder carcinoma cells has been molecularly cloned. The transforming sequences have been located within a 4.6 kilo base pair (kbp) DNA fragment that transforms NIH/3T3 cells with a specific activity of 5 X 10(4) focus-forming units/pmol. Homologous sequences present in normal human DNA have also been molecularly cloned. Comparison of the restriction endonuclease maps of the normal and transforming genes did not reveal any significant differences. These results suggest that subtle molecular changes are responsible for the acquisition of malignant properties by this gene in T24 cells. The T24 oncogene was found to be unrelated to transforming genes pre sent in a variety of human tumors other than bladder carcinomas. In contrast, the T24 oncogene is highly related to the onc genes of the BALB and Harvey strains of murine sarcoma viruses (MSVs). Preliminary characterization of the transcriptional and translational products of the T24 oncogene suggests that this gene is transcribed into a 1.2-kbp poly(A)-containing RNA whose translation yields a 23,000-dalton protein antigenically related to the transforming gene products of BALB and Harvey MSVs.

165. METABOLIC TURNOVER OF HUMAN C-RASH P21 PROTEIN OF EJ BLADDER CARCINOMA AND ITS NORMAL CELLULAR AND VIRAL HOMOLOGS

Ulsh LS, Shih TY
Laboratory of Molecular Oncology, National Cancer Institute, Frederick, Maryland.
Mol Cell Biol; 4(8):1647-52 1984

The EJ bladder carcinoma oncogene is activated by a point mutation in the c-rasH proto-oncogene at the 12th amino acid codon. In an attempt to understand the mechanism of oncogenic activation, a comparative study was undertaken to

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examine the metabolic turnover and subcellular localization of the p21 protein encoded by the EJ oncogene, the viral oncogene, and its normal cellular homolog. Pulse-labeling experiments indicated that both c-ras p21 proteins were synthesized by a very similar pathway, as was observed for the viral p21 protein of Harvey murine sarcoma virus. The pro-p21 proteins were detected in free cytosol, and the processed products were associated with plasma membrane. The intracellular half-life of p21 proteins was determined by pulse-labeling and chasing in the presence of excess unlabeled methionine. Although both p21 proteins of EJ and the normal c-ras genes which are not phosphorylated have a half-life of 20 h, the viral p21 protein of Harvey murine sarcoma virus which includes a phosphorylated form is much more stable in cells, having a half-life of 42 h, apparently due to phosphorylation.

166. PREDICTION OF THE THREE-DIMENSIONAL STRUCTURE OF THE TRANSFORMING REGION OF THE EJ/T24 HUMAN BLADDER ONCOGENE PRODUCT AND ITS NORMAL CELLULAR HOMOLOGUE

Pincus MR, van Renswoude J, Harford JB, Chang EH, Carty RP, Klausner RD

Laboratory of Biochemistry and Metabolism, National Institutes of Health, Bethesda, Maryland.

Proc Natl Acad Sci USA; 80(17):5253-7 1983

The three-dimensional structures of the transforming region of the product of the EJ/T24 human bladder oncogene and of the c-Ha ras-1 gene product have been calculated by using conformational energy calculations. These two genes, representing a transforming oncogene and its normal cellular homologue, encode 21,000-dalton peptides that differ by one amino acid at position 12. We therefore examined the energetically allowed conformations of the hydrophobic decapeptide surrounding this substitution site. The calculations show that the most favorable form of the c-Ha ras-1 gene product exists when glycine-12 is in a left-handed bend conformation. No other amino acid can adopt this conformation and thus the bladder oncogene peptide containing valine at position 12 has a markedly different three-dimensional structure. A simple model is proposed to account for the consequences of a position 12 mutation.

167. PREDICTED NUCLEOTIDE-BINDING PROPERTIES OF P21 PROTEIN AND ITS CANCER-ASSOCIATED VARIANT

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Laboratory of Chemical Physics, University of Groningen, The Netherlands.

Nature; 302(5911):842-4 1983

Recently, it has been demonstrated that a single point mutation is responsible for the acquisition of transforming properties by the EJ and T24 human bladder carcinoma gene. The point mutation consists of the conversion of guanine into thymine, which results in the replacement of a glycine by a valine at position 12 of the p21 protein encoded by the EJ and T24 genes. Sequence data of retroviral analogues of the p21 protein also indicate the importance for a glycine residue at position 12 in normal p21. Comparison of the sequence of the 37 N-terminal residues of the normal human p21 protein with the sequence of the dinucleotide-binding beta alpha beta unit in a group of structurally related enzymes, suggests that these residues of p21 fold into a very similar unit which is also involved in binding a nucleotide. We present here a three-dimensional model of the p21 beta alpha beta unit which explains directly why glycine at position 12 cannot be replaced by another residue without altering the nucleotide-binding properties of p21.

168. DIFFERENTIAL EXPRESSION OF P21RAS GENE PRODUCTS AMONG HISTOLOGICAL SUBTYPES OF FRESH PRIMARY HUMAN LUNG TUMORS

Kurzrock R, Gallick GE, Gutterman JU

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Cancer Res; 46(3):1530-4 1986

Activation of the ras oncogene has been associated with a number of human tumors. In this study, expression of p21ras in different histological types of fresh primary bronchogenic carcinomas was examined. p21ras products were detected in all lung tissues that were analyzed. Only 1 of 23 tumors demonstrated aberrant migration of p21ras. In contrast, 10 tumors had substantially elevated levels of p21ras products with respect to the adjacent normal lung tissues and with respect to the other lung tumors. There was no correlation between increased ras protein expression and tobacco exposure of the patient or extent of disease at the time of diagnosis. However, 9 of 11 tumors with a squamous component as opposed to only 1 of 12 tumors belonging to other histological classifications demonstrated increased p21ras. These data suggest that, in bronchogenic carcinomas, mutations associated with structural abnormalities and aberrant migration of p21ras occur infrequently as compared to quantitativ e changes in p21ras. Furthermore, differential expression of c-ras products in primary human lung tumors correlates with pathological cell type, and may be a common event in squamous cell carcinomas, but not adenocarcinomas or small cell carcinomas of the lung.

169. ALTERED GENE PRODUCTS ARE ASSOCIATED WITH ACTIVATION OF CELLULAR RASK GENES IN HUMAN LUNG AND COLON CARCINOMAS

Der CJ, Cooper GM

Sidney Farber Cancer Inst., Boston, MA, 02115

Cell; 32(1):201-208 1983

Two lung and two colon carcinoma cell lines of human origin, which contained the same activated rask transforming gene, expressed abnormal species of p21 proteins expressed in normal human cells and other human carcinomas. The abnormal species of p21 expressed by three of these cell lines were indistinguishable from each other, but differed from the abnormal p21 expressed by one lung carcinoma cell line. NIH cells transformed by DNAs of these carcinomas expressed the same abnormal p21 species, indicating that these abnormal proteins were encoded by the activated rask genes detected by transfection. These results indicate that transforming activity of rask genes in human lung and colon structure of their gene products, and that activation of rask genes can result from different molecular alterations in different individual neoplasms. (Author abstract) (36 Refs)

170. EXPRESSION OF P21RAS IN FRESH PRIMARY AND METASTATIC HUMAN COLORECTAL TUMORS

Gallick GE, Kurzrock R, Kloetzer WS, Arlinghaus RB, Gutterman JU

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Proc Natl Acad Sci USA; 82(6):1795-9 1985

Activation of the cellular oncogene ras has been implicated in many types of human malignancies. In this study, the relative levels of p21 protein product of ras (p21ras) in primary and metastatic colon tumors were compared to those in adjacent normal tissues. Nine of the 17 primary tumors had substantially elevated levels of p21ras with respect to adjacent normal tissues. Eight of these tumors were from Dukes' B and C stages. Four of the five tumors classified as 'D' stage

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(in which distant metastases are present) did not show elevated levels of p21ras. In metastases from primary colon tumors, nine of nine were considerably reduced in p21ras expression regardless of the site of metastasis. These data suggest that elevation of p21ras may be a common event in early stages of colon tumors, and tumor progression may lead to a more autonomous population of cells in which other growth factors supplant the role of this protein.

171. MONOCLONAL ANTIBODIES DEFINE DIFFERENTIAL RAS GENE EXPRESSION IN MALIGNANT AND BENIGN COLONIC DISEASES

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Laboratory of Tumor Immunology and Biology. National Cancer Institute. Bethesda. Maryland.

Nature: 311(5986):562-5 1984

DNAS of some human tumours can transform NIH 3T3 fibroblast cells, thus demonstrating the transforming potential of human ras genes (Hu-rasHa, Hu-rasKi, and Hu-rasN, respectively Harvey, Kirsten and neuroblastoma ras genes). Only a small percentage of a given type of human carcinoma, however, scores positive in this assay system. Activation of ras and subsequent transformation of NIH 3T3 cells are either by a point mutation in the ras gene or enhanced expression of the normal, or proto-onc, ras gene. If the transformation of a given human tumour involves the enhanced expression of the normal or cellular ras gene and the resulting gene product, the tumour DNA would probably score negative in the NIH 3T3 transfection assay. In human colon carcinoma, for example, lesions at position 12 of Hu-rasKi have been found. None of nine colon carcinomas obtained at biopsy, however, contain the ras lesion at this position, using a Hu-rasHa probe; one other colon carcinoma does appear to contain amplified proto-onc ras, and other colon carcinomas do have increased levels of ras RNA. There are at least three explanations for these observations. Either very few colon carcinomas contain point-mutated ras, the lesion in the majority of colon carcinomas is at a position other than 12 or ras activation in many colon carcinomas involves the enhanced expression of either the point-mutated or proto-onc form of a ras gene. We have now used monoclonal antibodies directed against a synthetic peptide reflecting sequences of the human T24 ras gene product to define ras p21 protein expression in a spectrum of colonic disease states. Immunohistochemical analyses of individual cells within tissue sections reveal differences in ras p21 expression in colon carcinomas compared with normal colonic epithelium, benign colon tumours and inflammatory or dysplastic colon lesions. Our data suggest that ras p21 expression is correlated with depth of carcinoma within the bowel wall, and is probably a relatively late event in colon carcinogenesis

172. EXPRESSION OF THE N-RAS ONCOGENE IN TUMORIGENIC AND NON-TUMORIGENIC HT 1080 FIBROSARCOMA X NORMAL HUMAN FIBROBLAST HYBRID CELLS (23 Refs)

Weissman BE. Mark CS. Benedict WF. Stanbridge EJ

Childrens Hospital-Los Angeles. California.

Prog Clin Biol Res: 175:141-9 1985

Keywords (MeSH): Animal; Cell Line; Cell Transformation, Neoplastic; Fibrosarcoma/*familial and genetic; Gene Expression Regulation; Hela Cells; Human; *Hybrid Cells; Mice; Mice, Nude; Neoplasm Proteins/genetics; Neoplasm Transplantation; *Oncogenes; Phosphoproteins/genetics; Review.

173. EXPRESSION OF KI-RAS ONCOGENE IN TUMOR CELL VARIANTS EXHIBITING DIFFERENT METASTATIC CAPABILITIES

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Department of Chemical Immunology. Weizmann Institute of Science. Beer-Sheva. Israel.

Int J Cancer: 35(2):227-30 1985

The expression of oncogenes in well-characterized B16 melanoma and UV-2237 fibrosarcoma cell variants that exhibit distinct metastatic properties was explored. The search for the expression of 11 different oncogenes revealed that the major oncogene in those two tumor systems is the Kirsten-ras (ki-ras). The results indicate that the amounts of specific ki-ras mRNA and the p21 protein are similar in both low- and high-metastatic counterparts. These results suggest that in these systems there is no apparent direct correlation between the amount and expression of the major cellular oncogene so far identified and the metastatic potential of these tumor cells.

174. ACTIVATION OF N-RAS IN A HUMAN MELANOMA CELL LINE

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Marie Curie Memorial Foundation, Research Institute, Oxted. Surrey. United Kingdom.

Mol Cell Biol: 5(3):582-5 1985

DNA isolated from cell line Mel Swift, a human melanoma cell line, transforms NIH3T3 cells. Southern blot analysis of DNA from secondary foci revealed conserved 8.8- and 7.8-kilobase EcoRI fragments which hybridized with a human repetitive sequence clone, blur 8. The activated transforming gene was identified as N-ras, and the 8.8-kilobase EcoRI fragment from a secondary transformant was cloned. Synthetic 17-mer oligonucleotides which spanned either the normal codon 61 (CAA) or a mutant codon 61 (AAA) were used for hybridization. Cloned N-ras from melanoma cell line Mel Swift hybridized to the mutant (AAA) oligonucleotide. From this we predicted a glutamine-to-lysine substitution in amino acid 61, a change confirmed by conventional sequencing of the first and second exons of N-ras from cell line Mel Swift. Transfection experiments showed that only those recombinant clones with the mutation in position 61 were biologically active.

175. ENHANCED EXPRESSION OF RAS ONCOGENE PRODUCT P21 IN HORMONE-DEPENDENT MAMMARY CARCINOMAS OF HUMANS AND RODENTS (MEETING ABSTRACT)

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Proc Annu Meet Am Assoc Cancer Res: 26:61 1985

The growth of hormone-dependent, DMBA-induced rat mammary carcinomas has been shown to be associated with the enhanced expression of a cellular proto-oncogene, r-ras(H). The 21,000-dalton ras gene product (p21) is a prominent in vitro translation product of mRNAs from the growing mammary carcinomas, and the translated p21 levels sharply decrease in tumors regressing upon hormone withdrawal (F.L. Huang and Y.S. Cho-Chung, Biochem. Biophy. Res. Commun. 123: 141, 1984). To assess whether the hormone-dependency of mammary tumors does, in fact, correlate with over-expression of c-ras gene, we now measured p21 levels in hormone-dependent vs hormone-independent rat mammary carcinomas and in human breast tumors characterized for estrogen and progesterone receptors. Immunoblotting analysis of p21 r evealed that the level of p21 in hormone-dependent rat tumors was about 7 fold that of hor-

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mone-independent tumors. The majority (30/50) of human breast carcinomas had high p21 levels, about 10-fold that of the normal breast tissue; 70% of these tumors were estrogen and progesterone receptor positive. p21 levels in the remaining 20/50 tumors were 3-fold that of the normal breast tissue, regardless of the receptor status. Fibroadenomas and fibrocystic tissue showed p21 levels similar to that of the normal mammary glands. Moreover, the high p21 levels in the mammary carcinomas correlated directly with high GTPase activity, as revealed by the photo-incorporation of 8-N3-[gamma-32P]GTP into the tumor lysates. These results suggested that the growth of hormone-dependent mammary carcinomas may correlate with quantitative activation of 'normal' p21 protein.

176. AMPLIFIED EXPRESSION OF P21 RAS PROTEIN IN HORMONE-DEPENDENT MAMMARY CARCINOMAS OF HUMANS AND RODENTS

DeBortoli ME. Abou-Issa H. Haley BE. Cho-Chung YS
Laboratory of Pathophysiology. National Cancer Institute. Bethesda. Maryland.
Biochem Biophys Res Commun: 127(2):699-706 1985

Western blotting analysis was utilized to determine the amount of a ras oncogene product, p21 present in mammary carcinomas of humans and rats. The levels of p21 in hormone-dependent rat tumors was about 7-fold that of hormone-independent tumors. The majority of human breast carcinomas examined had high p21 levels, about 10-fold that of the normal breast tissue; 70% of these tumors were estrogen and progesterone receptor positive. p21 levels in the remaining tumors were 3-fold that of the normal breast tissue, regardless of the receptor status. Fibroadenomas and fibrocystic disease showed p21 levels similar to that of the normal mammary glands. Moreover, the high p21 levels in the mammary carcinomas correlated directly with high GTPase activity, as revealed by the photo-incorporation of 8-N3-[gamma-32P]GTP into the tumor lysates. The results suggest that hormone-dependency of mammary carcinomas may correlate with quantitative change in 'normal' p21 protein.

177. HORMONE-REGULATED EXPRESSION OF CELLULAR RASH ONCOGENE IN MAMMARY CARCINOMAS IN RATS

Huang FL. Cho-Chung YS
Laboratory of Pathophysiology. National Cancer Institute. Bethesda. Maryland.
Biochem Biophys Res Commun: 123(1):141-7 1984

Differential gene expression has been observed in hormone-dependent rat mammary carcinomas during their growth and regression. A 22K MW protein, a prominent in vitro translation product of the growing as compared to the regressing tumor, was identified as the c-rasH-21,000-dalton transforming protein (p21) using a monoclonal antibody that reacts specifically with Harvey-related p21 species. The amount of p21-translated protein sharply decreased in the translation products of the regressing tumor s within 6 hours post ovariectomy or dibutyryl cyclic AMP treatment. The results show that an enhanced expression of the c-rasH oncogene is associated with hormone-dependent growth of mammary carcinomas in vivo and that suppression of this oncogene precedes the tumor regression induced by either hormone withdrawal or dibutyryl cyclic AMP treatment.

178. HIGH EXPRESSION LEVELS OF RAS P21 PROTEIN IN NORMAL MOUSE HEART TISSUES

Spandidos DA. Dimitrov T
Beatson Institute for Cancer Research. Glasgow. Scotland. U.K.
Biosci Rep: 5(12):1035-9 1985

We have investigated the levels of protein encoded by the ras oncogene in normal mouse tissues using an immunoblotting technique. We have found that heart from young or adult NIH or Balb C strains of mice contain high levels of ras protein as compared to lung, liver, spleen, kidney, brain and skeletal muscle tissues from the same animal. Our results indicate that cellular ras expression does not in every case correlate with cell proliferation.

179. EXPRESSION OF C-RAS TRANSCRIPTS AND P21 PROTEIN DURING LIVER REGENERATION AND HEPATOCARCINOGENESIS (MEETING ABSTRACT)

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Fed Proc: 44(5):1493 1985

The ras oncogenes are cellular DNA sequences thought to be involved in normal and neoplastic cell growth, but the function of these genes and the regulation of their expression is not understood. To compare c-ras expression during normal liver growth with liver carcinogenesis, we examined (1) the expression of ras H/K p21 protein during liver regeneration and (2) the levels of c-ras H/K transcripts in hepatocytes and oval cells purified from normal and preneoplastic rat livers and in cultured oval cells. We find that p21 is expressed in regenerating rat livers and that the expression of this protein increases with time after partial hepatectomy (PH). The level of p21 increases by 6 hr, peaks at 24 hr and declines by 72 hr. It is noteworthy that the max expression of p21 coincides with the peak of DNA synthesis 24 hr after PH and that the changes in levels of p21 correspond closely to the temporal changes in ras transcripts that we have previously shown during liver regeneration. During hepatocarcinogenesis levels of c-rasH transcripts are initially higher in hepatocytes than in oval cells whereas c-rasK transcripts are higher in oval cells. In cultured oval cells we find elevated levels of both c-rasH and c-rasK transcripts in quiescent and serum stimulated cells. These findings suggest transcriptional control of ras gene expression and provide further evidence of the importance of ras in normal and abnormal cell growth.

180. EXPRESSION OF RAS ONCOGENE P21 IN PROSTATE CANCER

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N Engl J Med: 314(3):133-7 1986

The major neoplastic transformation-inducing genes of human solid tumors are members of the ras oncogene family. We used an immunohistochemical assay to assess expression of both the unaltered and the mutated ras oncogene protein (p21) in normal and neoplastic prostatic cells. With the concentration of monoclonal antibody used in this study, epithelial and stromal cells from subjects with normal prostates and from 19 patients with benign prostatic hyperplasia were negative for p21 antigen. This antigen was detected in 2 of 6 prostates with Grade I carcinoma, 4 of 6 with Grade II, and all of 17 with higher grades. A semiquantitative immunohistochemical method demonstrated that expression of the p21 antigen in a carcinoma strongly correlated with nuclear anaplasia and was inversely related to the degree of glandular differentiation. However, markedly anaplastic tumors were often

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more heterogeneous in expression of p21 and contained areas of low staining for the antigen. Comparison of p21 antigen with tumor carcinoembryonic antigen and prostate-specific antigen demonstrated that ras p21 was the only phenotypic marker that correlated with histologic tumor grade. Thus, ras oncogene p21 may represent a new class of biologically relevant tumor markers and may be a useful adjunct to histopathologic examination in determining the prognosis of patients with prostate cancer.

181. ALTERATION OF THE GENOMES OF TUMOR CELLS

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Center for Cancer Research, Massachusetts Institute of Technology, Cambridge.
Cancer; 51(11):1971-5 1983

The transfer of DNA from tumor cells into normal cells has made possible the definition of oncogenes in the DNA of the donor tumor cells. Some of these oncogenes have been isolated by molecular cloning and found to derive from closely related normal cellular sequences. These normal antecedents are termed proto-oncogenes. Analysis of molecular clones of the proto-oncogene and its transforming allele indicate that the two genes are very similar. In one case the alteration of a single nucleotide in the normal gene resulted in the creation of an active oncogene. This point mutation affected a sequence encoding the 21,000 dalton protein, resulting in a glycine at its residue 12 being replaced by a valine. This altered protein mediates the resulting transformation of the cell. Such altered proteins are found in a number of lung and colon carcinomas. Although these oncogenes represent important determinants of the carcinogenic process, other genetic alterations appear to be necessary in order to achieve full conversion of a normal cell into a tumor cell.

182. EXPRESSION OF P21 RAS ONCOPROTEINS IN HUMAN CANCERS

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Cancer Res; 46(3):1465-70 1986

The expression of proteins encoded by ras oncogenes was examined in 52 fresh human tumors of 19 different types using antibodies generated to different peptide domains of ras proteins of molecular weight 21,000 (p21). Proteins related to ras genes were detected in 90% of tested tumors. In 21% of tumors there were high levels of ras p21 of greater than 40% of the level in a Harvey murine sarcoma virus transformed cell line. Predominance of either cellular Ha-ras or other ras p21s was found in 20 and 14% of tumors, respectively, and predominance of p21 other than Ha-ras was frequent in breast cancers. Abnormal electrophoretic mobility of p21 was observed in two cancers, and a novel rapidly migrating ras p21 was found in a rare malignant fibrohistiocytoma.

183. EXPRESSION OF P21RAS IN FRESH PRIMARY HUMAN LUNG TUMORS (MEETING ABSTRACT)

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Proc Annu Meet Am Assoc Cancer Res; 26:65 1985

Activation of cellular oncogenes belonging to the ras gene family has been implicated in several human malignancies by either differential expression of the normal proto-oncogene or by a point mutation. In this study, a monoclonal antibody (donated by Dr. E. Scolnick) which detects the p21 product of the c-ha-ras, c-Ki-ras, and N-ras genes was used to investigate ras expression in 19 paired samples of different histological subtypes of fresh primary human lung tumors and adjacent normal lung tissues. P21ras from lyophilized lung tissues (2.5 mg) was immunoprecipitated, then immunoblotted using the rat monoclonal antisera and reacted with 125I anti-rat IgG. P21ras was detected in all lung tissues examined. Only 1 of 19 (5.6%) lung tumors demonstrated aberrant migration of p21. In contrast, quantitative changes in p21ras were frequently observed, with 9 of 19 (47%) lung tumors demonstrating a greater than 3 fold elevation in p21 in the tumor with respect to the adjacent normal lung tissue. Tubulin levels (as determined by immunoblotting) were equivalent between each paired tumor and normal tissue. There was no correlation between ras expression and age or sex of the patient, history of tobacco use or stage of disease. Interestingly, 8 of 9 (89%) lung tumors with a squamous component in contrast to only 1 of 11 (9%) nonsquamous cell lung carcinomas demonstrated increased p21ras. These data suggest that differential expression of p21 in lung tumors correlates with histological classification and may be a consistent marker of squamous cell carcinomas but not adenocarcinomas or small cell carcinomas of the lung.

184. NUCLEOTIDE SEQUENCE OF THE RASHEED RAT SARCOMA VIRUS ONCOGENE: NEW MUTATIONS

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Science; 221(4606):555-7 1983

The nucleotide sequence of the oncogene of the Rasheed strain of rat sarcoma virus was determined. The oncogene (Ra-v-ras) encodes a 29,000-dalton (p29) transforming protein. This protein is distinct from the immunologically related 21,000-dalton protein (p21) of the Harvey murine sarcoma virus in its amino terminus and in having additional mutations in its carboxyl terminus. Although the functional significance of these changes is unknown, they appear to occur only in rat sarcoma virus.

185. COMPARATIVE BIOCHEMICAL PROPERTIES OF NORMAL AND ACTIVATED HUMAN RAS P21 PROTEIN

McGrath JP. Capon DJ. Goeddel DV. Levinson AD
Department of Molecular Biology, Genentech, Inc., San Francisco, California.
Nature; 310(5979):644-9 1984

Human Ha-ras1 cDNAs encoding normal and activated p21 polypeptides have been efficiently expressed in Escherichia coli and the biochemical activities associated with each polypeptide compared. In addition to the guanine nucleotide binding activity, normal p21 displays a GTPase activity which is selectively impaired by a mutation which activates its oncogenic potential.

186. THREE HUMAN TRANSFORMING GENES ARE RELATED TO THE VIRAL RAS ONCOGENES

Shimizu K. Goldfarb M. Suard Y. Perucho M. Li Y. Kamata T. Feramisco J. Stavnezer E. Fogh J. Wigler MH
Cold Spring Harbor Laboratory, New York.
Proc Natl Acad Sci USA; 80(8):2112-6 1983

Three distinct transforming genes present in human tumor cell lines are all related to the viral oncogenes of Harvey and Kirsten murine sarcoma viruses, designated v-H-ras and v-K-ras, respectively. The transforming gene of a bladder carcinoma cell line has been shown to be a human homolog to v-H-ras [Parada, L. F., Tabin, C. J., Shih, C. and Weinberg, R. A. (1982) Nature (London) 297, 474-478; Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S. and Barbacid, M. (1982) Nature (London) 298, 343-347]. The transforming gene common to one colon (SK-CO-1) and two lung carcinoma (SK-LU-1 and Calu-1) cell lines is the same human homolog

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of v-K-ras as is the transforming gene previously identified in a lung carcinoma cell line Lx-1 [Der, C. J., Krontiris, T. G. and Cooper, G. M. (1982) Proc. Natl. Acad. Sci. USA 79, 3637-3640]. The transforming gene of SK-N-SH neuroblastoma cells is weakly homologous to both v-H-ras and v-K-ras. NIH 3T3 cells transformed with the SK-N-SH transforming gene contain increased levels of a protein serologically and structurally related to the protein products of the v-H-ras and v-K-ras genes. Therefore, it represents a third member of the ras gene family, which we have called N-ras. Based on the homology with the v-ras genes, we have established the orientation of transcription and approximate coding regions of the cloned human K-ras and N-ras genes.

187. HOMOLOGIES BETWEEN SIGNAL TRANSDUCING G PROTEINS AND RAS GENE PRODUCTS

Hurley JB. Simon MI. Teplow DB. Robishaw JD. Gilman AG
Division of Biology. California Institute of Technology. Pasadena.
Science: 226(4676):860-2 1984

The guanosine triphosphate-binding proteins (G proteins) found in a variety of tissues transduce signals generated by ligand binding to cell surface receptors into changes in intracellular metabolism. Amino acid sequences of peptides prepared by partial proteolysis of the alpha subunit of a bovine brain G protein and the alpha subunit of rod outer-segment transducin were determined. The two proteins show regions of sequence identity as well as regions of diversity. A portion of the amino-terminal peptide sequence of each protein is highly homologous with the corresponding region in the ras protein (a protooncogene product). These similarities suggest that G proteins and ras proteins may have analogous functions.

188. PROTEINS ENCODED BY RAS ONCOGENES STIMULATE OR INHIBIT PHOSPHORYLATION OF SPECIFIC MITOCHONDRIAL MEMBRANE PROTEINS

Backer JM. Weinstein IB
Comprehensive Cancer Center. Columbia University. New York. N.Y. 10032.
Biochem Biophys Res Commun: 135(1):316-22 1986

We have examined the effects of a series of purified p21 proteins encoded by ras oncogenes and synthesized in E. coli via recombinant DNA methods, on the phosphorylation of proteins associated with isolated rat liver mitochondria. We find that these proteins markedly enhance the phosphorylation of a 36KD protein and inhibit phosphorylation of a 17KD protein. The phosphorylated residues on the 36KD protein are hydrolyzed by mild acid, suggesting that they involve phosphoamide bonds. These results suggest that p21 proteins may play a role in vivo by altering the phosphorylation of certain proteins.

189. AUTOPHOSPHORYLATION OF V-HA-RAS P21 IS MODULATED BY AMINO ACID RESIDUE 12

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Proc Natl Acad Sci USA. 81(19):2674-8 1984

The 21,000-dalton protein (p21) encoded by the ras oncogene of Harvey murine sarcoma virus (v-Ha-ras) becomes phosphorylated (pp21) in vivo and in vitro on threonine residue 59. p21 molecules encoded by cellular ras genes (c-Ha-ras-1) contain an alanine at position 59, and thus these p21 molecules are not phosphorylated. In this investigation, recombinant ras genes have been constructed between the 5' p21 coding region of normal (EC) or oncogenically activated (EJ) human c-Ha-ras-1 and the 3' p21 coding region of v-Ha-ras to generate p21 molecules containing a threonine phosphoacceptor site at position 59 and a glycine (EC/v-Ha) or valine (EJ/v-Ha) at residue 12. In transformed NIH 3T3 mouse fibroblast cells labeled with [35S]methionine, the ratio of pp21 to p21 was strikingly modulated by the amino acid at residue 12. v-Ha-ras p21 has an arginine at position 12, and 24% of total p21 was in the phosphorylated form. A glycine at residue 12 decreased the amount of pp21 to 14% of total p21, and a valine at residue 12 dramatically increased this value to 50%. In vitro, the valine form of p21 had 2.4- and 2.7-fold greater autophosphorylating activity than the glycine and arginine forms of p21, respectively, using [gamma-32P]GTP as phosphate donor, but the three p21 species had similar Km values for GTP (0.20-0.27 microM). These results indicate that a biochemical activity of p21 distinguishes between previously observed biological differences of normal and activated human ras genes.

190. CELL-FREE SYSTEM STUDIES ON THE PHOSPHORYLATION OF THE 17,000-20,000 DALTON PROTEIN INDUCED BY PHORBOL ESTER IN HUMAN LEUKEMIC CELLS AND EVIDENCE FOR A SIMILAR EVENT IN VIRALLY TRANSFORMED MURINE FIBROBLASTS

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Cancer Res: 45(7):3243-51 1985

We have shown previously that a prominent early signal in the phorbol-12-myristate-13-acetate (PMA) effect on leukemic cells as well as on other malignant cells is a rapid and dramatic increase in the turnover of phosphate in a Mr 17,000 to 20,000 cytosolic protein and a moderate increase in turnover of phosphate in a Mr 27,000 protein, as detected in the intact cells by 2-dimensional gel electrophoresis. To further elucidate the mechanism of this phosphorylation event, we have examined the protein kinases which can reconstitute this event in a cell-free system. Activation of the endogenous Ca2+-activated phospholipid-dependent protein kinase (Ca-PL-PK) as well as addition of purified Ca-PL-PK to the cytosol of HL-60 leukemic cells resulted in enhanced phosphorylation of phosphoprotein Mr 27,000 (PP27) but did not affect the phosphorylation of phosphoprotein Mr 17,000 to 20,000 (PP17-20). In contrast, PP17-20 was heavily phosphorylated under cell-free conditions by the catalytic subunit of cAMP-dependent protein kinase (cAMP-PK). Exposure of intact cells to dibutyryl-cAMP resulted in increased phosphorylation of PP17-20. These conditions also enhanced the phosphorylation of PP27, showing that PP27 can act as a substrate for both Ca-PL-PK and cAMP-PK under cell-free conditions. Tryptic digest analysis of PP17-20 showed that one of four phosphopeptides is preferentially phosphorylated in PMA-induced PP17-20. An additional phosphopeptide was phosphorylated in cAMP-PK-catalyzed PP17-20. Thus, cAMP-PK alone mimics the effect of PMA on phosphorylation of PP17-20, but it introduces additional modifications. The precise role of this kinase in PMA-induced phosphorylation of PP17-20 remains to be clarified. We found further that enhanced phosphorylation of PP17-20 is also associated with malignant transformation of NIH/3T3 cells transformed by V-rasKi oncogene of Kirsten sarcoma virus. The tryptic phosphopeptide map of PP17-20 (phosphorylated in vivo) in the transformed cells was similar to that of PP17-20 in PMA-treated HL-60 cells but not to that induced by cAMP-PK, suggesting that the process activated by PMA which leads to phosphorylation of PP17-20 resembles an intrinsic cellular process which is enhanced in certain malignant cells.

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191. REVERSAL OF TRANSFORMED PHENOTYPE BY MONOCLONAL ANTIBODIES AGAINST HA-RAS P21 PROTEINS

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Department of Molecular Oncology, Hoffmann-La Roche Inc., Roche Research Center, Nutley, NJ.
Exp Cell Res; 162(2):363-71 1986

The transforming activities of p21 ras proteins have been determined by micro-injection of these proteins into NIH3T3 cells. In order to facilitate functional studies on the effect of ras proteins on malignant transformation and normal cellular growth, analysis has been made with three monoclonal antibodies (YA6-172, Y13-238 and Y13-259) as originally reported by Furth et al. (J virol 43 (1982) 294). Purified immunoglobulin of Y13-259 has the highest titer of binding to bacterially synthesized p21 ras proteins. Experimental analyses indicate that only Y13-259 antibody will neutralize the transforming activity of the co-injected bacterially synthesized ras protein and the neutralization effect was blocked by co-injection of excess ras protein. In addition, micro-injection of Y13-259 immunoglobulin into transformed NIH3T3 cells (obtained by DNA transfection of NIH3T3 cells with molecularly cloned ras gene) reversed their transformed phenotypes. These results indicate that both bacterially synthesized p21 ras proteins and the natural ras proteins produced in NIH3T3 cells were neutralized by Y13-259 antibody.

192. INHIBITION OF GROWTH FACTOR-INDUCED DIFFERENTIATION OF PC12 CELLS BY MICROINJECTION OF ANTIBODY TO RAS P21

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Nature; 319(6055):680-2 1986

The protein products (p21) of the ras cellular proto-oncogenes are thought to transduce membrane signals necessary for the induction of cell division. However, there is uncertainty as to the precise role of ras p21 in mediating ligand-membrane receptor signals leading to cell differentiation. Treatment of rat phaeochromocytoma cells (PC12) with nerve growth factor (NGF) results in the induction of a number of phenotypic characteristics of sympathetic neurones, including cessation of cell division and outgrowth of neuronal processes (neurites). Here we report that microinjection of antibody to ras p21 into PC12 cells inhibited neurite formation and resulted in temporary regression of partially extended neurites, an effect which was observed up to 36 h after initiation of NGF treatment. Neurite formation induced by cyclic AMP was unaffected by injection of anti-p21 antibody. These results indicate that p21 is involved in the initiation phase of NGF-induced neurite formation in PC12 cells and has a role in hormone-mediated cellular responses distinct from cell proliferation.

193. SPONTANEOUS ACTIVATION OF A HUMAN PROTO-ONCOGENE

Santos E, Reddy EP, Pulciani S, Feldmann RJ, Barbacid M
Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland.
Proc Natl Acad Sci USA; 80(15):4679-83 1983

It has been recently shown that malignant activation of the c-has/bas proto-oncogene in T24 human bladder carcinoma cells was mediated by a single point mutation. A deoxyguanosine located at position 35 of the first exon of this proto-oncogene was substituted by thymidine. These findings predicted that the resulting oncogene would code for a structurally altered p21 protein containing valine instead of glycine as its 12th amino acid residue. We now report the spontaneous activation of the human c-has/bas proto-oncogene during transfection of NIH/3T3 cells. As in T24 cells, this in vitro activated oncogene also acquired malignant properties by a single point mutation. In this case we have detected a G leads to A transition, which occurred at the same position as the mutation responsible for the activation of the T24 oncogene. These results predict that the p21 protein coded for by the spontaneously activated c-has/bas gene will incorporate aspartic acid as its 12th amino acid residue. Computer analysis of the secondary structure of c-has/bas encoded p21 proteins indicates that substitution of the glycine residue located at position 12, not only by aspartic acid or valine but also by any other amino acid, would result in the same structural alteration. These findings indicate that a specific conformational change is sufficient to confer transforming properties to this p21 protein. Moreover, they predict that any mutation affecting the coding properties of the 12th codon of the c-has/bas proto-oncogene will lead to its malignant activation.

194. BIOLOGICAL AND BIOCHEMICAL PROPERTIES OF HUMAN RASH GENES MUTATED AT CODON 61

Der CJ, Finkel T, Cooper GM
Department of Pathology, Harvard Medical School, Boston, Massachusetts.
Cell; 44(1):167-76 1986

Using site-directed mutagenesis, we have introduced mutations encoding 17 different amino acids at codon 61 of the human rasH gene. Fifteen of these substitutions increased rasH transforming activity. The remaining two mutants, encoding proline and glutamic acid, displayed transforming activities similar to the normal gene. Overall, these mutants vary over 1000-fold in transforming potency. Increased levels of p21 expression were required for transformation by weakly transforming mutants. The mutant proteins were unaltered in guanine nucleotide binding properties. However, all 17 different mutant proteins displayed equivalently reduced rates of GTP hydrolysis, 8- to 10-fold lower than the normal protein. There was no quantitative correlation between reduction in GTPase activity and transformation, indicating that reduced GTP hydrolysis is not sufficient to activate ras transforming potential.

195. 31P-NMR SPECTRA OF THE HA-RAS P21.NUCLEOTIDE COMPLEXES

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Biochem Biophys Res Commun; 125(2):549-55 1986

Phosphorus nuclear magnetic resonance spectra of the Ha-ras oncogene product p21 and its nucleotide complexes have been obtained. It is shown that the 31P nuclear magnetic resonance spectra of a number of nucleotide-enzyme complexes show some common features. In particular, the chemical shift values of the beta-phosphorus resonance of enzyme-bound NTP and NDP (N = A, G) of hydrolases exhibit a downfield shift virtually identical for myosin, elongation factor Tu, and the Ha-ras oncogene product p21. This suggests that the stereochemistry around the beta-phosphorus might be similar in these compounds.

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196. PROTEIN STRUCTURE AND ONCOGENESIS (MEETING ABSTRACT)

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Molecular Basis of Cancer: An Interdisciplinary Discussion on Basic and Applied Aspects of Cancer, May 30-June 2, 1984, Buffalo, New York, Roswell Park Memorial Institute, p. 10, 1984.

It is known that the oncogene cloned from EJ bladder carcinoma cell lines transforms normal NIH 3T3 cells into malignant cells. This oncogene is different in only one base pair in the twelfth coding triplet from the corresponding proto-oncogene from normal human bladder tissue (GGC-GTC) which does not transform NIH 3T3 cells. The level of gene expression for both genes is the same, indicating that the 21,000 MW (p21) oncogene-encoded protein product (in which Val substitutes for Gly at position 12) is responsible for the malignant transformation. This finding raises the question as to whether this single amino acid substitution causes significant structural changes in the protein product. Using conformational energy calculations, we have computed the allowed conformations for the transforming region of the p21 protein product, involving residues 6-15. The sequence of these residues is Leu-(Val)3-Gly-Ala-Gly-Gly-Val-Gly for the proto-oncogene-encoded protein. The substitution in the oncogene-encoded protein is Val for Gly at position 12. We find that for its global minimum, the 'normal' protein adopts the alpha helical conformation for residues 6-10. This structure is then broken by a chain reversal involving Ala 11 and Gly 12, the latter residue adopting a left-handed twist conformation (D state) which no L-amino acid can adopt. The abnormal protein in its global minimum adopts an alpha-helical conformation for residues 6-11, terminating in a chain reversal at residues Val 12-Gly 13. The 'normal' protein (with Gly 12) can also adopt this conformation which is of slightly higher energy than that for the global minimum. Comparison of the two global minima demonstrates a major but confined structural difference in residues 11-13. The unique D conformation for Gly at position 12 suggests that any substitution of an L-amino acid at this position should result in a different structure that may cause malignant transformation. Furthermore, the fact that the normal protein can assume the same conformation as that for the abnormal protein suggests that at sufficiently high concentrations, the normal protein product may induce malignant transformation. Both of these predictions have been verified. It is known that random substitutions of L-amino acids including Val, Lys, Ser, and Asp for Gly all result in malignant transformation. Furthermore, when the normal proto-oncogene is spliced with a retro-viral LTR, the transfected gene causes large amounts of 'normal' protein to be synthesized. The cells containing this elevated level of protein are likewise transformed. The transforming region of the protein containing Gly 12 is quite flexible and adopts many low energy conformations. The protein containing Val 12, on the other hand, adopts only three basic conformations, all of which are determined by backbone-backbone interactions. If these interactions likewise predominate for any other amino acids substituting at position 12, the same conformations may also exist for these peptides, suggesting the possibility of a 'malignancy-causing' structure. Further, the presence of a D amino acid at position 12 may produce a completely normal protein because it would always promote the conformation at position 12. (1 Ref)

197. VIRAL HARVEY RAS P21 EXPRESSED IN ESCHERICHIA COLI PURIFIES AS A BINARY ONE-TO-ONE COMPLEX WITH GDP

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J Biol Chem; 260(7):3906-9 1985

The ras oncogene of Harvey murine sarcoma virus encodes a 21,000-dalton protein, p21, which mediates transformation. Viral Harvey ras p21, cloned into Escherichia coli HB101 lambda/pRAS1, has been purified to homogeneity by a three-step procedure. The purified E. coli p21 can be bound by a monoclonal antibody to viral Harvey ras p21 and has an amino acid composition consistent with that predicted from its DNA sequence. Purified E. coli p21 has been shown by HPLC analysis on an ion-exchange column to contain near stoichiometric amounts of GDP. This noncovalently associated GDP is seen in the UV absorption spectrum of the purified protein. The noncovalently associated GDP acts as a competitive inhibitor of the interaction of added guanine nucleotides with p21.

198. EXPRESSION OF NORMAL AND TRANSFORMING H-RAS GENES IN ESCHERICHIA COLI AND PURIFICATION OF THEIR ENCODED P21 PROTEINS

Lacal JC, Santos E, Notario V, Barbacid M, Yamazaki S, Kung H, Seamans C, McAndrew S, Croul R

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Proc Natl Acad Sci USA; 81(17):5305-9 1984

The H-ras gene of the BALB murine sarcoma virus (BALB-MSV) was placed under the transcriptional control of the tightly regulated PL promoter of bacteriophage lambda in the expression vectors pEV-vrf-1 and pRC23. Upon derepression of the PL promoter, large amounts (10-20% of total cellular protein) of the H-ras gene product p21 are synthesized in Escherichia coli. We constructed three H-ras gene expression vectors, designated pJCL-H5, pJCL-E30, and pJCL-33. pJCL-H5 directs the synthesis of p21, a fusion protein whose four amino-terminal residues are replaced by eight amino acids coded for by plasmid sequences. The 13 5' coding nucleotides of the BALB-MSV H-ras gene missing in pJCL-H5 were regenerated in pJCL-E30 by inserting a pair of complementary synthetic oligodeoxynucleotides. As a result, pJCL-E30 encodes a p21 protein, p21T, of sequence identical to that of the transforming p21 protein of BALB-MSV. pJCL-33 is a derivative of pJCL-E30 in which the 12th codon, AAA, a lysine codon, was replaced by GGA, a glycine codon. Thus, pJCL-33 directs the synthesis of a p21 protein, p21N, whose sequence corresponds to that of a normal cellular p21 protein. We report the purification of H-ras p21 proteins to apparent homogeneity by a method involving solubilization with chaotropic agents followed by reverse-phase high-performance liquid chromatography.

199. HIGH-LEVEL EXPRESSION OF ONCOGENE AND RETROVIRAL PROTEINS IN BACTERIA (MEETING ABSTRACT)

Lautenberger J, Court D, Kan N, Virgilio L, Samuel K, Josephs S, Wong-Staal F, Papas T

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XXIIth Symposium for Comparative Research on Leukemia and Related Diseases, July 7-11, Hamburg, p. 54, 1985.

A plasmid, pJL6, has been developed that has enabled us to express several oncogene and retróviral proteins in Escherichia coli at a high level. Presently the oncogenes mht, myb, myc, ras, and sis as well as a portion of the HTLV-I enve-

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lope have been expressed by the use of this system. pJL6 incorporates the strong well-regulated phage lambda pL promoter and the efficient translation initiation site and first 13 codons of the phage lambda cII gene. For each of the proteins, the size is close to that predicted from DNA sequence. Thus the E coli expression system provides an independent confirmation of the presence and length of open reading frames. Antibodies raised against the expressed v-myc gene immunoprecipitate the MC29 p110gag-myc in the Q8 nonproducer viral-transformed quail line. Th e expressed v-ras protein is recognized by monoclonal antibodies directed against the Harvey sarcoma virus p21ras, binds GDP, and is capable of autophosphorylation. The HTLV-I envelope protein expressed in bacteria is recognized by antibodies in patient serum.

200. PRODUCTION OF ONCOGENE-SPECIFIC PROTEINS AND HUMAN T-CELL LEUKEMIA (LYMPHOTROPIC) RETROVIRUS (HTLV-I) ENVELOPE PROTEIN IN BACTERIA AND ITS POTENTIAL FOR USE IN HUMAN CANCERS AND SEROEPIDEMIOLOGICAL SURVEYS

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Cancer Res: 45(9 Suppl):4568s-4573s 1985

The oncogenes coding for the Harvey murine sarcoma virus p21ras protein as well as those coding for myc, myb, and mht products were fused to the amino-terminal portion of the bacteriophage lambda cII gene on the expression vector pJL6. In addition two regions of the gene for the human T-cell leukemia virus subgroup I (HTLV-I) envelope were expressed in our bacterial system. Each of 11 human sera tested that had been shown to contain antibodies to HTLV-I or -II by an enzyme-linked immunosorbent assay recognized the bacterially synthesized envelope proteins. No reaction was detected when 17 control sera were tested. This system will be useful for large-scale seroepidemiological surveys for HTLV-I and related human retroviruses. The other oncogene products expressed in our bacterial vector system also demonstrated specific immunoreactivities. In addition to this feature the bacterial ras protein was seen to bind guanosine diphosphate and was capable of autophosphorylation. Taken together these data suggest that the proteins produced with high efficiency by the bacterial expression system can be immunologically recognized as antigens and can in part perform some of their associated biochemical functions.

201. HIGH-LEVEL EXPRESSION IN ESCHERICHIA COLI OF ENZYMATICALLY ACTIVE HARVEY MURINE SARCOMA VIRUS P21RAS PROTEIN

Lautenberger JA. Ulsh L. Shih TY. Papas TS
Laboratory of Molecular Oncology. National Cancer Institute. Bethesda. Maryland.
Science: 221(4613):858-60 1983

The gene for the Harvey murine sarcoma virus (Ha-MuSV) p21ras protein was fused to the amino-terminal portion of the bacteriophage lambda cII gene on the expression vector pJL6. The fusion was such that transcription was controlled by the well-regulated phage lambda pL promoter, and translation initiated in the cII gene continued in frame into the ras gene sequences that code for p21. When the pL promoter was derepressed, the Escherichia coli cells harboring the fusion plasmid synthesized 23,000-dalton protein, which represented more than 10 percent of the total cellular protein. This protein was chimeric and contained 14 residues, which were specified by the vector; these residues were foll owed by all of the amino acids that make up Ha-MuSV p21ras except for four residues at the amino-terminal end. The protein appears similar to Ha-MuSV p21ras in that it undergoes immunoprecipitation by monoclonal antibodies directed toward that protein, binds guanosine diphosphate, and is capable of autophosphorylation.

202. PROCESSING AND FATTY ACID ACYLATION OF RAS1 AND RAS2 PROTEINS IN SACCHAROMYCES CEKEVISIAE

Fujiyama A. Tamanoi F
Cold Spring Harbor Laboratory. NY 11724.
Proc Natl Acad Sci USA: 83(5):1266-70 1986

We demonstrate the pathway for the biosynthesis of RAS1 and RAS2 gene products of Saccharomyces cerevisiae leading to their localization in membranes. The primary translation products of these genes are detected in a soluble fraction. Shortly after synthesis, these precursor molecules are converted to forms that migrate slightly faster than the precursor forms on a NaDodSO4/polyacrylamide gel. These processed proteins are further modified by fatty acid acylation, which is detected by [3H]palmitic acid labeling. The acylated derivatives are found exclusively in cell membranes, indicating the translocation of the RAS proteins from cytosol to membranes during maturation process. The attached fatty acids can be released by mild alkaline hydrolysis, suggesting that the linkage between the fatty acid and the protein is an ester bond. The site of the modification by fatty acid is presumably localized to the COOH-terminal portion of the RAS proteins. Fractionation of the membranes by sucrose gradient demonstrates that a majority of the fatty-acylated RAS proteins are localized in plasma membrane.

203. A YEAST GENE ENCODING A PROTEIN HOMOLOGOUS TO THE HUMAN C-HAS/BAS PROTO-ONCOGENE PRODUCT

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Nature: 306(5944):704-7 1983

Organisms amenable to easy genetic analysis should prove helpful in assessing the function of at least those proto-oncogene products which are highly conserved in different eukaryotic cells. One obvious possibility is to pursue the matter in Drosophila melanogaster DNA, which has sequences homologous to several vertebrate oncogenes. Another is to turn to the yeast Saccharomyces cerevisiae, if it contains proto-oncogene sequences. Here we report the identification of a gene in S. cerevisiae which codes for a 206 amino acid protein (YP2) that exhibits striking homology to the p21 products of the human c-has/bas proto-oncogenes and the transforming p21 proteins of the Harvey (v-rasH) and Kirsten (v-rasK) murine sarcoma viral oncogenes. The YP2 gene is located between the actin and the tubulin gene on chromosome VI and is expressed in growing cells. The protein it encodes might share the nucleotide-binding capacity of p21 proteins.

204. MAMMALIAN AND YEAST RAS GENE PRODUCTS: BIOLOGICAL FUNCTION IN THEIR HETEROLOGOUS SYSTEMS

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Science: 228(4696):179-84 1985

Activated versions of ras genes have been found in various types of malignant tumors. The normal versions of these genes are found in organisms as diverse as mammals and yeasts. Yeast cells that lack their functional ras genes,

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RASSC-1 and RASSC-2, are ordinarily nonviable. They have now been shown to remain viable if they carry a mammalian rasH gene. In addition, yeast-mammalian hybrid genes and a deletion mutant yeast RASSC-1 gene were shown to induce morphologic transformation of mouse NIH 3T3 cells when the genes had a point mutation analogous to one that increases the transforming activity of mammalian ras genes. The results establish the functional relevance of the yeast system to the genetics and biochemistry of cellular transformation induced by mammalian ras genes.

205. CHARACTERIZATION AND DEVELOPMENTAL EXPRESSION OF A DROSOPHILA RAS ONCOGENE

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Mol Cell Biol; 5(4):885-9 1985

We cloned a Drosophila melanogaster ras gene (Dmras64B) on the basis of its homology to the ras oncogen from Harvey murine sarcoma virus. This gene mapped at chromosomal position 64B on the left arm of the third chromosome. Sequencing of Dmras64B revealed extensive amino acid homology with the proteins encoded by the human and Saccharomyces cerevisiae ras genes. The coding region of the Drosophila gene is interrupted by two introns located in different positions with respect to its human counterpart. Dmras64B encodes three different RNAs (1.6, 2.1, and 2.6 kilobases long) that are constantly expressed throughout the development of the fly.

206. EXPRESSION OF RAS CELLULAR ONCOGENES DURING DEVELOPMENT OF DROSOPHILA MELANOGASTER

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Mol Cell Biol; 5(6):1540-2 1985

The transcription patterns of three v-Ha-ras-related cellular oncogenes in Drosophila melanogaster were studied. Each gene coded for at least two distinct transcripts. The larger transcript of each gene was expressed at a similar abundance during the entire life cycle of fruit flies, whereas the shorter transcripts were much more abundant in embryonic stages than at later stages.

207. ONCOGENE EXPRESSION IN A RADIATION INDUCED RAT THYROID CARCINOMA (MEETING ABSTRACT)

Kaminsky S, Mukaby T, Zain S
Cancer Center, University of Rochester Medical School
Proc Annu Meet Am Assoc Cancer Res; 26:65 1985

Cancer induction is recognized as the single most important somatic effect of exposure of population s to low doses of ionizing radiation. The objective of the current study is to investigate the role of oncogene expression in radiation carcinogenesis. We selected to work with an in vivo rat thyroid carcinogenesis model because of the exquisite sensitivity of the rat thyroid gland to the carcinogenic effect of radiation and the pathobiological similarities between human and rat thyroid neoplasms. Tissue derived from an undifferentiated thyroid carcinoma (TTRKD), which arose at a transplant site grafted 2 years previously with irradiated (500 cGy) thyroid cells, was utilized as a source of tumor material for analysis of oncogene expression. Untreated thyroid tissue and hyperplastic tissue were used as normal and proliferative controls, respectively. Dot blot analysis revealed the presence of mRNAs which hybridized with cDNA probes of 4 oncogenes, myc, ras k, ras H, and abl in normal and hyperplastic thyroid tissue as well as tissue from TTRKD. Hybridizations using 6 other cDNA oncogene probes failed to detect any mRNA transcripts. While expression of the 4 oncogenes is quantitatively similar, Southern analysis of DNA has shown rearrangements in the DNA restriction maps for ras k, m/c and abl oncogenes in TTRKD relative to normal tissue. Preliminary experiments analyzing the poly A+-RNA suggest a size difference in the RNA coding for the p21 protein of ras k. The potential role of these modifications in TTRKD DNA and RNA in radiation induced thyroid carcinogenesis will be discussed.

208. STRUCTURE AND FUNCTION OF RAS AND BLYM ONCOGENES

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Hamatol Bluttransfus; 29:273-6 1985

Keywords (MeSH): Animal; Base Sequence; *Cell Transformation, Neoplastic; Cells, Cultured; Chickens; Human; Lymphoma/familial and genetic; Mice; Mice, Inbred Strains; Neoplasm Proteins/genetics; Neoplasms/familial and genetic/microbiology; *Oncogenes; Receptors, Endogenous Substances/genetics; Retroviridae/genetics; Transfection; Transferrin/genetics; Viral Proteins/genetics.

2.

sis protein product

209. THE V-SIS/PDGF-2 TRANSFORMING GENE PRODUCT LOCALIZES TO CELL MEMBRANES BUT IS NOT A SECRETORY PROTEIN

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EMBO J; 4(7):1783-92 1985

The v-sis transforming gene encodes the woolly monkey homologue of human platelet-derived growth factor (PDGF) polypeptide 2. After its synthesis on membrane bound polyribosomes, the glycosylated precursor dimerizes in the endoplasmic reticulum and travels through the Golgi apparatus. At the cell periphery, the precursor is processed to yield a dimer structurally analogous to biologically active PDGF. Small amounts of two incompletely processed forms are detectable in tissue culture fluids of simian sarcoma virus (SSV) transformants. However, the vast majority remains cell associated. Thus, th is growth factor-related transforming gene product is not a classical secretory protein. These findings define possible cellular locations where the transforming activity of the sis-PDGF-2 protein may be exerted.

210. ANTISERA TO A SYNTHETIC PEPTIDE OF THE SIS VIRAL ONCOGENE PRODUCT RECOGNIZE HUMAN PLATELET-DERIVED GROWTH FACTOR

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Nature; 307(5947):180-3 1984

It has recently been reported that the sequences of the sis oncogene of simian sarcoma virus (SSV) and of human platelet-derived growth factor (PDGF) are very similar, establishing the most solid link yet between the mitogenic actions of growth factors and the transforming proteins of retroviruses. To investigate molecular mechanisms of transformation I have produced antisera against synthetic peptides corresponding to segments of the protein sequences predicted by the nucleotide sequences of viral oncogenes. Applying this approach to the case of sis and PDGF, I report here the results of probing outdated human platelets with an antiserum directed against a synthetic peptide representing residues

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139-155 of the predicted sequence of the SSV transforming protein, p28sis (ref. 3). I detected peptides of apparent molecular weights (MWs) 30,000 to 31,000 (30-31K) and 16-18K, which correspond to the apparent molecular weights of nonreduced and reduced PDGF. In addition, a peptide of MW 21,000 was detected in platelets and a protein of MW 56,000 was detected in SSV-infected marmoset cells.

211. EVIDENCE THAT THE V-SIS GENE PRODUCT TRANSFORMS BY INTERACTION WITH THE RECEPTOR FOR PLATELET-DERIVED GROWTH FACTOR

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Science; 230(4723):327-30 1985

A scheme for partial purification of biologically active v-sis-coded protein from cells transformed with simian sarcoma virus (SSV) has made possible a functional comparison of the transforming protein with platelet-derived growth factor (PDGF). The SSV-transforming gene product is capable of specifically binding PDGF receptors, stimulating tyrosine phosphorylation of PDGF receptors, and inducing DNA synthesis in quiescent fibroblasts. Each of these activities was specifically inhibited by antibodies to different regions of the v-sis gene product. Moreover, viral infection of a variety of cell types revealed a strict correlation between those cells possessing PDGF receptors and those susceptible to transformation by SSV. These findings provide evidence that SSV-transforming activity is mediated by the interaction of a virus-coded mitogen with PDGF receptors.

212. DETECTION OF C-SIS TRANSCRIPTS AND SYNTHESIS OF PDGF-LIKE PROTEINS BY HUMAN OSTEOSARCOMA CELLS

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Science; 226(4677):972-4 1984

Platelet-derived growth factor (PDGF) has been previously shown to be homologous to the transforming gene of simian sarcoma virus (v-sis), and inappropriate expression of the cellular counterpart of the v-sis gene (c-sis) has been implicated in the generation of mesenchymal tumors. The U-2 OS human osteosarcoma line was shown to contain multiple c-sis transcripts. Immunoprecipitation experiments with antiserum to PDGF identified a variety of polypeptides ranging in size from 18,000 to 165,000 daltons that were immunoprecipitated specifically from U-2 OS cell extracts. The osteosarcoma also was shown to secrete a 29,000-dalton protein having the serological and structural characteristics of PDGF.

213. ACTIVATED HUMAN MONOCYTES EXPRESS THE C-SIS PROTO-ONCOGENE AND RELEASE A MEDIATOR SHOWING PDGF-LIKE ACTIVITY

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Nature; 319(6049):158-60 1986

Current ideas about the mechanism of wound healing and the pathogenesis of atherosclerosis, pulmonary fibrosis and hepatic fibrosis suggest a central role for the mononuclear phagocyte in attracting and/or stimulating the proliferation of mesenchymal cells. We demonstrate here that activated human blood monocytes, but not resting monocytes, release a mediator that attracts smooth muscle cells and cooperates with other mediators to stimulate fibroblast proliferation. This mediator is very similar to platelet-derived growth factor (PDGF): its chromatographic properties and chemical stability are similar to those of PDGF, it competes with 125I-PDGF for binding to fibroblasts and it immunoprecipitates with anti-PDGF antibodies. In parallel, stimulated monocytes, but not resting monocytes, express the c-sis proto-oncogene, a gene coding for one of the PDGF chains, consistent with the concept that expression of the c-sis proto-oncogene may be involved in the ability of mononuclear phagocytes to modulate the accumulation of mesenchymal cells.

214. THE SIS-GENE ACTION IN HUMAN SARCOMA CELLS (MEETING ABSTRACT)

von der Helm K. Born M. Wernicke D
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XV Meeting of the European Tumour Virus Group, September 19-24, 1984, Urbino, Italy, p. 124, 1984.

The oncogene of the simian sarcoma virus is the v-sis-gene; its gene product is a polypeptide of 28 kd which can be precipitated by an anti C-terminal-sis-peptide serum. The cellular sis-sequences, c-sis, probably code for the platelet-derived growth factor, PDGF, which also can be precipitated by the anti-sis-serum. In several cell lines derived from human primary sarcoma biopsies we found a prot ein, similar in mol wt to p28, also precipitable by the anti-sis-serum. In two original human sarcoma biopsies we found evidence that the sis-gene has undergone a rearrangement. Whether this rearrangement is responsible for an activation of the sis expression in human sarcomas was discussed.

215. REQUIREMENT FOR A SIGNAL SEQUENCE IN BIOLOGICAL EXPRESSION OF THE V-SIS ONCOGENE

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Science; 226(4679):1197-9 1984

The protein encoded by the simian sarcoma virus oncogene (v-sis) contains a signal sequence, derived from the envelope gene of the parental retrovirus, which is required for transformation. Removal of the proposed signal sequence was correlated with loss of biological activity. This activity was restored to inactive deletion mutants by fusion with the coding region for a heterologous signal sequence. Biological activity of v-sis was also abolished by either a small deletion within the coding region of the signal sequence or by a point mutation introduced by site-directed mutagenesis.

216. BLOCKADE OF AUTOCRINE STIMULATION IN SIMIAN SARCOMA VIRUS-TRANSFORMED CELLS REVERSES DOWN-REGULATION OF PLATELET-DERIVED GROWTH FACTOR RECEPTORS

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Proc Natl Acad Sci USA; 81(23):7466-70 1984

The viral (v)-sis oncogene encodes a protein (p28sis) that is structurally homologous to platelet-derived growth factor (PDGF). We have shown that simian sarcoma virus (SSV)-transformed cells containing the v-sis oncogene release a Mr 20,000 substance that is recognized by antisera to synthetic peptide sequences contained in p28sis. Medium conditioned by SSV-transformed cells competes with 125I-labeled PDGF for specific PDGF receptor sites, initiates DNA synthesis, and stimulates tyrosine phosphorylation of the PDGF receptor when added to normal cells. When normal cells are cocultured with SSV-transformed cells, the PDGF receptors of the normal cells are down-regulated by factors released from the transformed cells. Thus, SSV-transformed cells release

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material that is functionally similar to PDGF. We have used anti-phosphotyrosine antibodies to purify PDGF receptors and to detect PDGF-stimulated receptors in normal cells. SSV-transformed cells have no PDGF receptors detectable by these antibodies or by 125I-labeled PDGF binding studies. However, when SSV-transformed cells are exposed to suramin, a compound that blocks binding of PDGF to its receptors, the receptors reappear on the cell surface and within 8 hr are present at the same levels as in control cells. These 'new' receptor sites can be phosphorylated in response to PDGF. Thus, the absence of PDGF receptors in SSV-transformed cells is due to down-regulation of the receptors by an autocrine mechanism that can be blocked by suramin.

217. CHARACTERIZATION OF A PHOSPHOPROTEIN DETECTED BY SIS-SPECIFIC ANTIBODIES (MEETING ABSTRACT)

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XXIIth Symposium for Comparative Research on Leukemia and Related Diseases, July 7-11, Hamburg, p. 82, 1985.

The c-sis gene codes for a polypeptide of 28 kd which is processed to a smaller polypeptide of about 11 kd that is identical to the B-chain monomer of the platelet derived growth factor (PDGF) dimer. The simian sarcoma virus (SSV) v-sis gene product bears structural and immunological homology with the c-sis gene product. With antibodies against two oligopeptides, one representing the c-terminal amino acid sequence of v-sis, the other representing the internal amino acid sequence of B-chain PDGF, we could immunoprecipitate the p28sis protein from SSV-transformed cell extracts. From most of these SSV transformed cell extracts, both anti-peptide sera were capable of immunoprecipitating a 115kd phosphoprotein in addition to the p28sis. The 115 kd protein is phosphorylated but could also be phosphorylated in vitro by gamma8-P32-ATP. The in vitro phosphorylation was independent of c-AMP or Ca++. This 115 k polypeptide was detected also and at comparable levels in actively growing non-transformed human fibroblasts. Maintenance of transformed cells in medium containing only 0.5% serum did not influence the production of the phosphorylated 115 k polypeptide whereas in non-transformed cells kept in low serum-medium the amount of phosphorylated 115 k polypeptide was markedly reduced.

218. DETECTION OF E. COLI COLONIES EXPRESSING THE V-SIS ONCOGENE PRODUCT WITH MONOCLONAL ANTIBODIES MADE AGAINST SYNTHETIC PEPTIDES

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Department of Human Genetics, University of Pennsylvania, School of Medicine, Philadelphia 19104.
J Immunol Methods: 85(1):169-82 1985

A method for immunodetection of individual epitopes on eukaryotic proteins synthesized in E. coli colonies is described. The system is developed using monoclonal antibodies produced against the Ha-ras protein produced in E. coli JM103. Monoclonal antibodies made against a synthetic peptide from the v-sis oncogene sequence are then used to identify bacterial colonies in which the v-sis protein is being produced. Production of the v-sis protein by these E. coli colonies was confirmed by immunoblot analysis. The assay utilizes peroxidase conjugated anti-mouse immunoglobulin and 4-chloro-1-naphthol to detect the positive colonies and can detect on the order of 200 pg antigen per E. coli colony.

219. PLATELET-DERIVED GROWTH FACTOR AND DOUBLE-STRANDED RIBONUCLEIC ACIDS STIMULATE EXPRESSION OF THE SAM E GENES IN 3T3 CELLS

Zullo JN. Cochran BH. Huang AS. Stiles CD
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Cell: 43(3 Pt 2):793-800 1985

Platelet-derived growth factor (PDGF) stimulates expression of a 'competence' gene family in Balb/c-3T3 cells. The competence family contains the c-myc and c-fos genes together with several functionally uncharacterized genes (JE, KC, and r-fos) that have been isolated as cDNA clones. We show that double-stranded ribonucleic acid is a potent inducer of the competence gene family. Infection with vesicular stomatitis virus also induces expression of this gene family. Conversely, PDGF stimulates expression of genes hitherto characterized as responsive to double-stranded ribonucleic acids, including the beta-fibroblast interferon and (2'-5')-oligoadenylate synthetase genes. These PDGF-inducible genes could conceivably function in a feedback loop to control 3T3 cell growth. Some of the genes, such as c-fos and c-myc, are induced quickly by PDGF and may initiate a round of cell division. Others, such as beta-fibroblast interferon and (2'-5')-oligoadenylate synthetase, are induced more slowly and may function as feedback inhibitors of the growth response to PDGF.

220. CULTURED HUMAN ENDOTHELIAL CELLS EXPRESS PLATELET-DERIVED GROWTH FACTOR B CHAIN: CDNA CLONING AND STRUCTURAL ANALYSIS

Collins T. Ginsburg D. Boss JM. Orkin SH. Pober JS
Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115.
Nature: 316(6030):748-50 1985

Vascular endothelial cells have a central role in various pathophysiological responses such as acute inflammation, wound healing and atherogenesis. The anatomical position of endothelial cells between blood leukocytes and the surrounding vascular smooth muscle cells or stromal fibroblasts may intensify and focus the effects of released endothelial cell products. Endothelial cells in culture produce a platelet-derived growth factor (PDGF)-like mitogen. PDGF purified from platelets is a basic protein with an apparent relative molecular mass (Mr) of approximately 30,000 (reviewed in refs 2, 3) and is believed to comprise two polypeptide chains, PDGF-A and PDGF-B (also referred to as PDGF-1 and PDGF-2; refs 5, 6). Sequence analysis of PDGF B chain has revealed a striking homology with the predicted sequence of p28sis, the transforming protein of simian sarcoma virus. sis-Homologous transcripts have been detected by Northern blot analysis of RNA from cultured endothelial cells. However, there are no structural data available on either the protein product or the messenger RNA to establish the identity of the endothelial-derived mitogen with either chain of PDGF. Here we report the isolation and complete sequence analysis of a sis-homologous complementary DNA clone from human endothelia l cells, providing an opportunity to study the structure of sis as transcribed by a normal (untransformed) cell. Our results establish that normal human endothelial cells in culture express the B chain of PDGF, and that endothelial-derived PDGF B chain is synthesized as a predicted precursor polypeptide of Mr 27,281.

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221. GROWTH FACTORS. A NEW DIMENSION IN UNDERSTANDING ONCOGENESIS

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Klin Wochenschr; 63(16):740-6 1985

The current understanding in biology and function of 4 growth factors is reviewed. PDGF suggests functions for proto-oncogens in normal cells, which may interact in tightly linked hierachies to induce malignant growth. PDGF-requirement of normal fibroblast cell-lines is lost when the cells are infected with tumor viruses. TGF is able to stimulate growth of normally anchorage dependent cells in an anchorage independent manner in soft agar. This ability is thought to be the best in-vitro correlate of neoplastic transformation. The peptide hormones bombesin/gastrin releasing factor and EGF can act as autocrine growth factors in various lung cancer cell-lines and stimulate clonal tumor cell growth in-vitro. The potential clinical application of these types of growth factors may enable the in-vitro growth from any lung cancer patient and allow individual drug testing. TCGF produced by T-cells to activate T-cells, is central to immune stimulation and immune response. Models for potential indirect anticancer effects either by in-vivo administration or by in-vivo incubation plus passive transfer of T-cells are presented to be initiated in future clinical trials.

C.
Cytoplasmic Products of Cellular Oncogenes: Additional Reports

222. ONCOGENE AND ITS PRODUCTION OF AN AVIAN SARCOMA VIRUS Y73

Toyoshima K, Yoshida M, Kawai S, Kitamura N, Yamamoto T, Segawa K

The Institute of Medical Science, University of Tokyo, Japan.

Int Symp Princess Takamatsu Cancer Res Fund; 12:275-83 1982

The Y73 strain of avian sarcoma virus isolated from a transplantable chicken tumor was defective in its replicating capacity. The virus caused sarcoma but not acute leukosis in chickens even when inoculated intravenously. It induced transformed cell-foci in cultured fibroblasts and the viral genome responsible for in vitro transformation was 26S RNA. The RNA was composed of sequences in common with helper virus RNA and Y73-specific sequence. The specific sequence 'yes' did not hybridize with complementary DNA to the src gene of Rous sarcoma virus (cDNAsrc) and it had a unique counterpart in normal cell DNA. The yes gene was located in the middle of the 26S genome and the sequences common to the helper virus were located toward both ends. The 26S RNA coded for an polyprotein of 90,000 daltons (p90) which included p19 of viral core proteins in addition to the polypeptide unique to the yes gene. p90 had protein kinase activity specific for tyrosine residue and itself could be phosphorylated at tyrosine residue in vivo and in vitro, and at serine residue in vivo.

223. THE HUMAN MET ONCOGENE IS RELATED TO THE TYROSINE KINASE ONCOGENES

Dean M, Park M, Le Beau MM, Robins TS, Diaz MO, Rouley JD, Blair DG, Vande Woude GF

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Nature; 318(6044):385-8 1985

The met oncogene was previously isolated from a chemically transformed human cell line, MNNG-HOS. Recent evidence has demonstrated that two classes of transcripts are expressed from the met proto-oncogene locus. The met oncogene, however, expresses an aberrant RNA which has sequences in common with both transcripts. We now report partial nucleotide sequencing of the human met oncogene and show that met is related to the protein kinase oncogenes and growth factor receptors. The met nucleotide sequence is not identical to that of any published gene, and it is more closely homologous to the tyrosine kinases than to the serine/threonine kinases. Within the tyrosine kinase family, the sequenced met domains are most closely related to the human insulin receptor and the viral abl gene. In situ chromosome hybridization has mapped met to human chromosome 7 band 7q21-q31, a location distinct from that of other kinases. This is also a region associated with nonrandom chromosomal deletions observed in a portion of patients with acute nonlymphocytic leukaemia. The accompanying paper shows that this chromosomal locus is also tightly linked with the human heredity disease cystic fibrosis.

224. IDENTIFICATION AND PREFERENTIAL EXPRESSION IN THYMIC AND BURSAL LYMPHOCYTES OF A C-ETS ONCOGENE-ENCODED MR 54,000 CYTOPLASMIC PROTEIN

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Proc Natl Acad Sci USA; 83(6):1714-8 1986

The avian retrovirus E26 is unique among acute leukemia viruses in its ability to induce transformation of cells belonging to either the myeloid or erythroid lineage. The genome of E26 carries two oncogenes, v-myb and v-ets, that are derived from distinct cellular loci, c-myb and c-ets. We have constructed a plasmid vector that allows expression of part of the coding region of v-ets in a bacterial host. Antisera to the bacterially synthesized ets protein specifically precipitated the E26-encoded P135gag-myb-ets transforming protein. These antisera permitted us to identify a chicken c-ets-encoded protein of Mr 54,000 (P54c-ets) that shares 7 out of 10 of its major [35S]methionine-containing tryptic peptides with the v-ets-encoded domain of P135gag-myb-ets. Unlike P135gag-myb-ets and the Mr 75,000 translation product of c-myb (P75c-myb), which are nuclear proteins, P54c-ets was found to be predominantly cytoplasmic. P54c-ets is expressed at low levels in most cell lines and tissues tested, including bone marrow cells and circulating lymphocytes. P54c-ets, together with a minor but closely related Mr 56,000 protein, was found to be expressed at high levels in chicken thymocytes and bursal lymphocytes.

225. THE ETS SEQUENCE FROM THE TRANSFORMING GENE OF AVIAN ERYTHROBLASTOSIS VIRUS, E26, HAS UNIQUE DOMAINS ON HUMAN CHROMOSOMES 11 AND 21: BOTH LOCI ARE TRANSCRIPTIONALLY ACTIVE

Watton DK, McWilliams-Smith MJ, Nunn MF, Duesberg PH, O'Brien SJ, Papas TS

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Proc Natl Acad Sci USA; 82(21):7294-8 1985

Human DNA segments homologous to the ets region from the transforming gene of avian erythroblastosis virus, E26, were molecularly cloned and shown to be closely related to the viral equivalent by hybridization and partial sequence analysis. The transforming gene of E26 has a tripartite origin with the structure delta gag [1.2 kilobases (kb) from the viral gag gene]-myb(0.9 kb from the chicken myb gene)-ets (1.6 kb from the chicken ets gene). Human ets DNA is located on two distinct human chromosomes. The human ets-1 locus

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on chromosome 11 encodes a single mRNA of 6.8 kb; the second locus, ets-2 on chromosome 21, encodes three mRNAs of 4.7, 3.2, and 2.7 kb. The ets-related sequences of human DNA on chromosomes 11 and 21 are discontiguous, except for a small overlap region encoding 14 amino acids, where 12 are conserved between these two loci. By contrast, the chicken homolog has contiguous ets-1 and ets-2 sequences and is primarily expressed in normal chicken cells as a single 7.5-kb mRNA. We conclude that the ets sequence shared by the virus, the chicken, and humans is likely to contain at least two dissociable functional domains, ets-1 and ets-2. Thus, the tripartite transforming gene of E26 includes four distinct domains that may be functionally relevant for the transforming function of the virus (delta gag, myb, ets-1, and ets-2).

226. **A LYMPHOCYTE-SPECIFIC PROTEIN-TYROSINE KINASE GENE IS REARRANGED AND OVEREXPRESSED IN THE MURINE T CELL LYMPHOMA LSTRA**

Marth JD. Peet R. Krebs EG. Perlmutter RM
Howard Hughes Medical Institute. Seattle. Washington.
Cell; 43(2 Pt 1):393-404 1985

Protein-tyrosine kinases are implicated in the control of normal and neoplastic cell growth. We have used molecular cloning strategies to characterize a lymphocyte-specific protein-tyrosine kinase gene distinct from but closely related to src and yes. This gene, encoded by a genetic locus defined here as lskT, is rearranged and overexpressed in the murine T cell lymphoma LSTRA. Thus alterations in the structure or expression of this protein-tyrosine kinase gene may in some cases mediate neoplastic transformation. In addition, transcription of the normal lskT gene is restricted to cells of lymphoid origin. We infer that the lskT-encoded protein-tyrosine kinase may aid in transducing proliferative or differentiative signals unique to lymphocytes.

227. **DETECTION AND CHARACTERIZATION OF THE PROTEIN ENCODED BY THE V-REL ONCOGENE**

Rice NR. Copeland TD. Simek S. Oroszlan S. Gilden RV
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Virology; 149(2):217-29 1986

To identify the protein encoded by v-rel, the oncogene of reticuloendotheliosis virus (REV-T), antisera have been raised to three synthetic peptides derived from the translation of our previously published v-rel DNA sequence [R.M. Stephens, N.R. Rice, R.R. Hiebsch, H.R. Bose, Jr., and R.V. Gilden, Proc. Natl. Acad. Sci. USA 80, 6229-6233 (1983)]. Sera to all three peptides precipitate a 59,000 Da protein from REV-T-transformed chicken lymphoid cells. This protein is not detectable in uninfected chick embryo fibroblasts, and its observed size is in good agreement with the 56,000 Da predicted by the DNA sequence. We conclude that this protein is the v-rel product and designate it p59rel. To search for evidence of post-translational processing of this protein, cells were grown in the presence of glycosylation inhibitors. These resulted in no detectable difference in the size of p59rel. Nor was its size detectably altered during the course of a pulse-chase experiment. Growth of cells in the presence of [32P] orthophosphate, however, revealed that p59rel is a phosphoprotein. It is also closely associated with a protein kinase activity, for precipitation with one of the peptide antisera (but not the other two) resulted in strong kinase activity in the immune complex pellet. During this reaction, p59rel itself becomes phosphorylated. Kinase activity was retained in the immune complex following detergent and high salt washes, leaving open the possibility that p59rel is itself a kinase.

228. **ONCOGENE EXPRESSION IN RETICULOENDOTHELIOSIS VIRUS-TRANSFORMED LYMPHOID CELL LINES AND AVIAN TISSUES**

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Department of Microbiology. University of Texas at Austin 78712-1095.
J Virol; 57(1):371-5 1986

The genome of reticuloendotheliosis virus (REV-T) contains a unique oncogene, designated v-rel, which is inserted into the env region. Employing a cloned rel DNA probe, a single 2.9- to 3.0-kilobase v-rel mRNA was identified in poly(A)+ RNA from REV-T-transformed lymphoid cell lines. A 4.0-kilobase rel-specific transcript corresponding to the cellular homolog of the v-rel oncogene was identified in MSB-1 cells, a herpesvirus-transformed lymphoid cell line. Cytodot hybridization was used to quantitate the levels of rel, c-rel, c-myc, c-myb, c-abl, c-fms, c-Ha-ras, c-Ki-ras, c-src, c-yes, c-mos, and c-sis mRNA in REV-T-transformed cells. The levels of rel transcription in REV-T-transformed cells were elevated only two to eightfold over levels found in the transformed immature avian lymphoid cell line MSB-1. The relatively modest levels of rel transcription in REV-T-transformed cells and the significant differences between the lengths of the v-rel and c-rel mRNA suggest that REV-T transformation is the result of the production of an altered rel protein. The c-rel proto-oncogene is expressed in all avian hematopoietic tissues but is not expressed at significant levels in brain and muscle. The transcription of other proto-oncogenes is not enhanced in REV-T-transformed lymphoid cell lines.

229. **A NEW ACUTE TRANSFORMING FELINE RETROVIRUS AND RELATIONSHIP OF ITS ONCOGENE V-KIT WITH THE PROTEIN KINASE GENE FAMILY**

Besmer P. Murphy JE. George PC. Qiu FH. Bergold PJ. Lederman L. Snyder HW Jr. Brodeur D. Zuckerman EE. Hardy WD
Laboratory of Molecular Oncology. Memorial Sloan-Kettering Cancer Center. New York. New York.
Nature; 320(6061):415-21 1986

A new acute transforming feline retrovirus, the Hardy-Zuckerman 4 feline sarcoma virus (HZ4-FeSV), has been isolated from a feline fibrosarcoma. The viral genome of HZ4-FeSV contains a new oncogene designated v-kit, has the structure 5' delta gag-kit-delta pol-delta env 3' and specifies a gag-kit polyprotein of relative molecular mass 80,000. The predicted kit amino-acid sequence displays partial homology with tyrosine-specific protein kinase oncogenes. HZ4-FeSV appears to have been generated by transduction of feline c-kit sequences with feline leukaemia virus.

D.

Nuclear Protein Products of Cellular Oncogenes

1.

myb protein product

230. **IDENTIFICATION OF THE LEUKEMOGENIC PROTEIN OF AVIAN MYELOBLASTOSIS VIRUS AND OF ITS NORMAL CELLULAR HOMOLOGUE**

Boyle WJ. Lipsick JS. Reddy EP. Baluda MA
University of California. School of Medicine. Los Angeles.
Proc Natl Acad Sci USA; 80(10):2834-8 1983

The genome of the replication-defective avian myeloblastosis virus (AMV) contains an inserted cellular sequence (amv) that is part of the oncogene responsible for acute myeloblastic leukemia in chickens infected with AMV. Three antisera

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raised against distinct synthetic peptides predicted from the long open reading frame of amv specifically precipitated the same 48-kilodalton protein (p48amv) from leukemic myeloblasts but not from normal hematopoietic tissue, fibroblasts, or from fibroblasts infected with the AMV helper virus, MAV-1 (myeloblastosis-associated virus type 1). p48amv is not glycosylated or phosphorylated and does not appear to act as a protein kinase in vitro. The same three antisera that recognized p48amv also specifically precipitated a common 110-kilodalton protein from normal uninfected hematopoietic tissue. This normal cellular homologue of the AMV leukemogenic protein , p110proto-amv, was not present in normal fibroblasts, MAV-1 infected fibroblasts, or, interestingly, in some leukemic myeloblasts. We conclude that p48amv is the leukemogenic product of an altered, transduced, partial protooncogene. Short helper-virus sequences provide its carboxyl terminus and also may provide the amino terminus.

231. THE PRODUCT OF THE RETROVIRAL TRANSFORMING GENE V-MYB IS A TRUNCATED VERSION OF THE PROTEIN ENCODED BY THE CELLULAR ONCOGENE C-MYB

Klempnauer KH. Ramsay G. Bishop JM. Moscovici MG. Moscovici C. McGrath JP. Levinson AD
George W. Hooper Foundation. San Francisco. California.
Cell: 33(2):345-55 1983

Avian myeloblastosis virus (AMV) is an oncogenic retrovirus that rapidly causes myeloblastic leukemia in chickens and transforms myeloid cells in culture. AMV carries an oncogene, v-myb, that is derived from a cellular gene, c-myb, found in the genomes of vertebrate species. We constructed a plasmid vector that allows expression of a portion of the coding region for v-myb in a procaryotic host. We then used the myb-encoded protein produced in bacteria to immunize rabbits. The antisera obtained permitted identification of the proteins encoded by both v-myb and chicken c-myb. The molecular weights of the products of v-myb and c-myb (45,000 and 75,000 respectively) indicate that the v-myb protein is an appreciably truncated version of the c-myb protein.

232. CHARACTERIZATION OF THE V-MYB ONCOGENE PRODUCT (MEETING ABSTRACT)

Boyle WJ. Lampert MA. Li A. Ong J. Baluda MA
Dept. of Pathology. UCLA Sch. of Medicine. Los Angeles. CA 90024
XXIIth Symposium for Comparative Research on Leukemia and Related Diseases. July 7-11. Hamburg. p. 67. 1985.

Avian myeloblastosis virus (AMV) induces acute myelogenous leukemia in chickens and transforms myeloid cells in vitro. The oncogene of AMV, v-myb, generates a subgenomic viral m-RNA of 2.0 kb that contains at its 5' end a short (presumably 6 codons) viral gag sequence and at its 3' end the twelve 3' terminal codons, including the termination codon, of the viral env gene. The leukemogenic product of v-myb is a nuclear phosphoprotein of 48,000 Mr with serine as the primary phosphoacceptor. Phosphorylation of tyrosine was not detected and neither was protein kinase activity. In AMV-transformed leukemic myeloblasts p48 has a half-life of 30 min, suggesting that it may act as a regulatory factor in nuclear processes. Analysis of synchronized leukemic cells revealed that either the synthesis of p48 is enhanced or that p48 is stabilized just prior to DNA synthesis (S phase). To elucidate its leukemogenic role we have fractionated nuclei from leukemic cells and isolated distinct subnuclear fractions which contain p48. After specific immunoprecipitation with anti-p48 antisera we have detected 47% of p48 in the nucleoplasm, 4% in the chromatin fraction and 49% in the residual lamina-nuclear matrix fraction. Our findings suggest that p48 may be part of a complex located at the lamin-chromatin junction that is involved in DNA replication and/or transcription.

233. THE TRANSFORMING GENE OF AVIAN MYELOBLASTOSIS VIRUS (AMV): NUCLEOTIDE SEQUENCE ANALYSIS AND IDENTIFICATION OF ITS TRANSLATIONAL PRODUCT

Papas TS. Rushlow KE. Watson DK. Bader JP. Ray D. Reddy EP
Hamatol Bluttransfus: 28:207-13 1983

The genome of the avian myeloblastosis virus (AMV) has undergone a sequence substitution in which a portion of the region normally coding for the env protein has been replaced by cellular sequences. We have determined the complete nucleotide sequence of this region. Examination of the AMV oncogenic sequence revealed an open reading frame starting with the initiation codon ATG and terminating with the triplet TAG within the acquired cellular sequences and terminating with the triplet TAG at a point thirty-three nucleotides into helper viral sequences to the right of the helper-viral-cellular junction. The stretch of 795 nucleotides would code for a protein of 265 amino acids with a molecular weight of 30,000 daltons. The eleven amino acids at the carboxy terminus of such a protein would be derived from the env gene of helper virus. Antibodies were prepared against synthetic peptides derived from the predicted amino acid sequences. One such antibody precipitated two proteins of apparent nucleotide weight of 30,000 daltons and 51,000 daltons.

234. SUBCELLULAR LOCALIZATION OF PROTEINS ENCODED BY ONCOGENES OF AVIAN MYELOBLASTOSIS VIRUS AND AVIAN LEUKEMIA VIRUS E26 AND BY CHICKEN C-MYB GENE

Klempnauer KH. Symonds G. Evan GI. Bishop JM
G.W. Hooper Foundation. San Francisco. California.
Cell: 37(2):537-47 1984

Analysis of the subcellular location of the proteins encoded by the oncogenes of avian myeloblastosis virus and avian leukemia virus E26 (p45v-myb and p135gag -myb-ets, respectively) and by the chicken c-myb gene (p75c-myb) shows that all three proteins are located in the nucleus. In AMV-infected (but not transformed) chicken fibroblasts p45v-myb also resides in the nucleus, indicating that a nuclear location of p45v-myb in these cells is not sufficient to achieve transformation. In AMV-transformed myeloblasts a small fraction of p45v-myb occupies an additional site in the perinuclear region of the cytoplasm. If the myeloblasts are caused to differentiate to macrophages, most of p45v -myb is found in the cytoplasm. This redistribution of p45v -myb within the cell may be responsible for reversion of the transformed phenotype.

235. DETECTION AND LOCALIZATION OF THE V-MYB(AMV) GENE PRODUCTS OF AVIAN MYELOBLASTOSIS VIRUS BY A SYNTHETIC PEPTIDE ANTISERUM

Wright SE. Smith DP. Wright DE
Viral Oncology Laboratory. Veterans Administration Medical Center. Salt Lake City. Utah 84148.
J Gen Virol: 66 (Pt 12):2755-66 1985

An antiserum made against a synthetic peptide from an internal region of the predicted amino acid sequence of the avian myeloblastosis virus (AMV) transforming v-myb(AMV) gene identified two products, p46v-myb(AMV) and p32v-myb(AMV), which were localized in the nucleus of AMV-transformed myeloblasts. We propose that these proteins are the in vivo products of the v-myb(AMV) gene and thus the transforming protein(s) of AMV.

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236. INDUCED DIFFERENTIATION OF AVIAN MYELOBLASTOSIS VIRUS-TRANSFORMED MYELOBLASTS: PHENOTYPIC ALTERATION WITHOUT ALTERED EXPRESSION OF THE VIRAL ONCOGENE

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Mol Cell Biol: 4(12):2587-93 1984

Cells of a clone of avian myeloblastosis virus-transformed myeloblasts were induced to differentiate to adherent myelomonocytic cells by treatment with lipopolysaccharide. These adherent cells were subcultured and maintained as a line for more than 6 months with lipopolysaccharide present. Cells of this line were induced to differentiate to nondividing macrophage-like cells by the addition of the tumor promoter 12-O-tetradecanoylphorbol-13-acetate. In this way, the following homogeneous cell populations representing three distinct stages of myeloid differentiation were obtained: I, actively dividing myeloblasts that grew in suspension: II, actively dividing adherent cells; and III, fully differentiated nondividing cells resembling macrophages. When the expression of v-myb (the oncogene of avian myeloblastosis virus) was examined in cells of these three differentiation stages, it was found that the protein encoded by v-myb (p45v-myb) continued to be synthesized in similar quantities and showed no obvious alteration (assessed by partial proteolytic digestion and two-dimensional gel electrophoresis) during differentiation. These results show that cells transformed by v-myb can be induced to differentiate without affecting the expression of v-myb and imply that, during differentiation, the effect of v-myb is suppressed by a mechanism other than altered expression of the oncogene.

2.

myc protein product

237. HUMAN N-MYC IS CLOSELY RELATED IN ORGANIZATION AND NUCLEOTIDE SEQUENCE TO C-MYC

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Nature. 319(6048):73-7 1986

N-myc, a cellular gene related to the c-myc proto-oncogene, was originally identified on the basis of its very frequent amplification and overexpression in a restricted set of tumours, most notably human neuroblastomas. That N-myc may have a causal role in the genesis of these tumours is suggested by the observation that in the rat embryo fibroblast co-transformation assay it has a transforming po tential similar to that of c-myc. The apparent structural and functional homology of N-myc and c-myc suggests that they may be members of the same protooncogene family. However, despite these apparent similarities, expression of the two genes appears to be dramatically different with respect to tumour specificity, as well as tissue and developmental stage specificity. To further elucidate the common and unique aspects of N-myc and c-myc gene structure and function in normal and transformed cells, we have determined the organization of human N-myc and the nucleotide sequence of its messenger product, and we report here that N-myc and c-myc have a similar intron/exon structure and that their protein products share regions of significant homology.

238. THE NUCLEAR ONCOGENE PRODUCTS MYC AND MYB (MEETING ABSTRACT)

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XXIIth Symposium for Comparative Research on Leukemia and Related Diseases. July 7-11. Hamburg. p. 55. 1985.

The viral oncogenes v-myc and v-myb and the human cellular homologue hu-c-myc have been expressed in bacteria. The bacterial proteins were isolated and served for antibody preparations and the isolation of a monoclonal antibody. Furthermore antisera against a variety of synthetic peptides were produced. These sera were used for indirect immunoprecipitation, fluorescence and immunoaffinity chromatography to define properties of oncogene products in virus-transformed cells and human tumor cell lines. In 15 tumor lines the level of expression and the size of the molecule vary. A 64,000 mol wt hu-c-myc protein was purified 3,000fold from Colo320 cells. It binds to double-stranded DNA in vitro and interacts with specific DNA restriction fragments. Peptide antibodies localize the DNA-binding domain in the carboxy-terminal half of the protein. The human c-myb protein was detected at low levels in a few human tumor lines. It is a molecule of 75,000 mol wt. v-myb and the E26 gag-myb-ets proteins were isolated and characterized in vitro. The purified proteins bind to DNA in vitro. DNA binding is thermolabile with proteins from E26 ts mutants (Moelling et al, Cell, in press). (1 Ref)

239. STUDIES ON THE INTERACTION OF THE HUMAN C-MYC PROTEIN WITH CELL NUCLEI: P62C-MYC AS A MEMBER OF A DISCRETE SUBSET OF NUCLEAR PROTEINS

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Cell: 43(1):253-61 1985

We have analyzed the localization of the human c-myc product (p62c-myc) at steady state in cells by immunoprecipitation and immunoblotting. We show that p62c-myc is extracted from nuclei by mild salt concentrations (below 200 mM), without affecting gross nuclear structure or causing extraction of major chromatin components. We observe no association between p62c-myc and the nuclear matrix. We also demonstrate that p62c-myc is a member of a discrete subset of nuclear proteins that are all rendered irreversibly insoluble in situ by exposure of isolated nuclei to physiological temperatures (37 degrees C). p62c-myc is sequestered into a similar insolubie complex in cells that have been subjected to heat shock. Finally, we show that avian v-myc and v-myb proteins in isolated nuclei also become insoluble after exposure to temperatures above 37 degrees C. We discuss the possible implications of these results.

240. EXPRESSION AND CHARACTERIZATION OF THE HUMAN C-MYC DNA-BINDING PROTEIN

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Mol Cell Biol: 5(3):448-56 1985

In an effort to study in detail the nature of the protein product of the human protooncogene c-myc, we have expressed the gene at high levels in Escherichia coli. The c-myc coding region was taken from a full-length cDNA clone and inserted into a vector designed to express foreign gene products efficiently in E. coli. Pulse-labeling experiments indicated that the rate of expression of c-myc in this thermoinducible expression system is very efficient. The product was relative-

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ly stable and accumulated to approximately 10% of total cellular protein. A purification protocol was devised which allowed the c-myc protein to be readily purified in quantities sufficient for detailed biochemical and physical analyses. A high-titer polyclonal antiserum was raised against the pure protein and shown to immunoprecipitate the p110gag-myc fusion protein of MC-29-infected quail cells. This antiserum also selectively detects a protein with an apparent molecular weight of 64,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis from a Burkitt lymphoma cell line. We conclude that this 64-kilodalton protein is the human c-myc gene product since the E. coli-made protein exhibits an equivalent molecular weight on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, even though its calculated molecular weight is 49,000. Furthermore, we demonstrate that the bacterially made human c-myc protein is a DNA-binding protein and that it exhibits a high nonspecific affinity for double-stranded DNA.

241. THE MYC PROTEINS ARE NOT ASSOCIATED WITH CHROMATIN IN MITOTIC CELLS

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EMBO J: 3(12):2947-50 1984

The protein products of cellular and viral myc oncogenes are detected in nuclei by immunofluorescence. No myc fluorescence is found in nucleoli. In mitotic cells the myc antigens are not found associated with metaphase chromosomes, but are diffusely distributed throughout the cytoplasm. Cytoplasmic myc fluorescence is first observed when chromatin begins to condense in early prophase. Granular nuc lear myc fluorescence is again discerned in telophase cells, when the nuclear envelope is formed and becomes more prominent upon cytokinesis; concomitantly the diffuse cytoplasmic myc staining is lost. These results suggest that myc proteins not only bind to DNA or chromatin, but are also associated with other structural systems in the nuclei.

242. IDENTIFICATION OF NUCLEAR PROTEINS ENCODED BY VIRAL AND CELLULAR MYC ONCOGENES

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George W. Hooper Foundation, University of California Medical Center, San Francisco.
Nature: 306(5940):274-7 1983

The myelocytomatosis viruses are a family of replication-defective avian retroviruses that cause a variety of tumours in chickens and transform both fibroblasts and macrophages in culture through the activity of their oncogene v-myc. A closely related gene (c-myc) is found in vertebrate animals and is thought to be the progenitor of v-myc. Changes in the expression and perhaps the structure of c-myc have been implicated in the genesis of avian, murine and human tumours (for a review, see ref. 15). Elucidation of the mechanisms by which v-myc and c-myc might elicit tumorigenesis requires identification of the proteins encoded by these genes. To this end, we have expressed a portion of v-myc in a bacterial host and used the resulting protein to raise antisera that react with myc proteins. We report here that v-myc and c-myc encode closely related proteins with molecular weights (MWs) of approximately 58,000. Integration of retroviral DNA near or within c-myc in avian lymphomas apparently enhances expression of the gene. Here we have used cells from one such tumour to identify the protein encoded by c-myc and find that the coding domain for the gene is probably intact.

243. NUCLEAR LOCALIZATION AND DNA BINDING OF THE TRANSFORMING GENE PRODUCT OF AVIAN MYELOCYTOMATOSIS VIRUS

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Nature: 296(5854):262-266 1982

A previous study has shown that the activated cellular gene in certain lymphomas was homologous to the oncogene of an acute avian leukemia virus, MC29. In MC29, the oncogene is fused to the viral structural gene, gag. The mol wt of the fused gene is 110,000 (110 K) and is designated as p110gag-myc. For further analysis, the 110 K protein was purified from the 35S-methionine-labeled MC29-transformed quail embryo fibroblast cell line MC29-Q8-NP (which does not produce virus particles, but expresses the transforming protein gag-myc) by an immune affinity column containing protein A-Sepharose to which monoclonal anti-p19 IgG was covalently coupled in conditions which link the constant region of the IgG molecule to the solid phase. Immunofluorescence studies and cell fractionation of MC29-trans formed fibroblasts indicate that the 110 K protein is predominantly located in the nucleus. The purified protein was found to bind to double-stranded DNA. These properties may be related to the role of the protein in transformation. (22 Refs)

244. NUCLEAR LOCALIZATION AND DNA BINDING PROPERTIES OF A PROTEIN EXPRESSED BY HUMAN C-MYC ONCOGENE

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Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts.
Science, 225(4663):718-21 1984

Antisera to the human cellular myc oncogene product were used to identify a human c-myc specific protein with a molecular weight of 65,000. Subcellular fractionation showed that the human c-myc protein is predominantly found in the cell nucleus. The p65Kc-myc protein binds to double- and single-stranded DNA as measured by a DNA affinity chromatography assay.

245. B CELL GROWTH FACTOR AND ANTI-MU ACTIVATE MYC ONCOGENE EXPRESSION IN HUMAN B CELLS (MEETING ABSTRACT)

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Yale Univ. Sch. of Med., New Haven, CT 06510
Fed Proc, 44(4):970 1985

We have recently shown that transcriptional expression of the cellular myc oncogene is activated in purified human B cells by B cell growth factor (BCGF) or anti-mu stimulation. Using the in situ hybridization technique, human B cells purified from peripheral blood were probed with 35S-labeled v-myc for myc-specific RNA at 0, 1/2, 2, 8, 24, 48, 72, and 120 hr of stimulation with mitogenic concentrations of anti-mu and/or BCGF. Estimates of relative amounts of myc-specific RNA were determined by counting grains over individual cells. At 2 hr the majority of cells showed a greater than 20-fold increase in myc expression compared to resting B cells. Elevated myc expression was present in some cells at all time points, but the fraction of cells with myc activation decreased over time from greater than 90% of cells at 2 hr to 30-70% of cells at 120 hr. Separation of a 72-hr culture of BCGF-stimulated cells into cells in G1, S, and G2 phases of the cell cycle by flow cytometry showed that myc expression was elevated in all 3 pre-mitotic phases and maximally expressed in S phase. Expression of erb-B, H-ras, and beta actin was unchanged after anti-mu or BCGF stimulation. These studies suggest that two different proliferative signals can activate

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myc expression in normal human B cells and that myc expression is increased in the pre-mitotic phase of the cell cycle.

246. IDENTIFICATION AND CHARACTERIZATION OF THE PROTEIN ENCODED BY THE HUMAN N-MYC ONCOGENE

Slamon DJ. Boone TC. Seeger RC. Keith DE. Chazin V. Lee HC. Souza LM
Department of Medicine. UCLA School of Medicine 90024.
Science; 232(4751):768-72 1986

The human N-myc gene is related to the c-myc proto-oncogene, and has been shown to have transforming potential in vitro. Many studies have reported amplification of N-myc in human neuroblastoma and re tinoblastoma cell lines. In primary tumors, amplification of the gene was found to correlate directly with behavior of the tumor. Specific restriction fragments of a partial complementary DNA clone of N-myc from LA-N-5 human neuroblastoma cells were placed into a bacterial expression vector for the purpose of producing antigens representative of the N-myc protein. Rabbits immunized with these antigens produced antisera that recognized a protein of 62-64 kilodaltons in neuroblastoma cells. By several criteria, this protein appears to be part of the same proto-oncogene family as the c-myc protein. Moreover, the antisera to fragments of this protein were capable of histochemically identifying malignant cells in clinical specimens.

247. CHARACTERIZATION OF C-MYC PROTEINS FROM AVIAN BURSAL LYMPHOMA CELL LINES

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Department of Microbiology. University of Virginia School of Medicine. Charlottesville 22908.
Virology; 150(1):178-86 1986

We have used a rabbit antiserum directed against a portion of the MC29 viral myc protein expressed in bacteria to characterize the cellular myc protein from three different avian bursal lymphoma cell lines (1104HI, 1104BI S13, BK25), and from normal chick embryo cells. The phosphorylated myc proteins immunoprecipitated from these cells varied in molecular weight from 58 to 62 kDa and localized to the cell nucleus, as shown by cell fractionation experiments. Pulse-chase experiments established that these proteins had short half-lives ranging from 12 min for the myc proteins from the 1104BI S13 cell line to 25 min for myc proteins from both the 1104HI and the BK25 cell lines. The structural relatedness of the proteins was established by comparing their partial proteolytic digestion products (Cleveland analysis) with the partial proteolytic digestion products of the MH2 viral myc protein. The anti-myc-serum also immunoprecipitated a 48-kDa protein from each of the bursal cell lines. We have identified this protein as a breakdown product of the bursal cell myc proteins. The different size and number of these bursal cell myc proteins may be a direct result of the specific site of integration as well as the orientation of the retrovirus LTR sequence relative to the adjacent cellular myc allele.

248. EXPRESSION OF C-MYC RNA IN BURSAL LYMPHOMA CELL LINES: IDENTIFICATION OF C-MYC-ENCODED PROTEINS BY HYBRID-SELECTED TRANSLATION

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Hutchinson Cancer Research Center. Seattle. Washington.
Cell; 33(2):335-44 1983

We examined expression of the c-myc locus in four cell lines established from bursal lymphomas induced by avian leukosis virus. In all four lines the level of myc-related RNA was elevated. In three lines a majority of the myc-containing RNAs lacked viral-LTR-related sequences, in contrast to results obtained with primary tumors. This suggests that LTR sequences are not required for maintenance of high level c-myc expression. One line, RP9, has a complex pattern of myc RNAs containing LTR sequences, and one of these RNAs is packaged into virions. Using hybrid selection of RNAs with myc DNA, followed by in vitro translation, we detected translation of myc-related proteins from RNA of all four cell lines. The sizes of these proteins differ among the cell lines. The major polypeptides detected were 64, 57, and 54 kilodaltons. Events leading to elevation of c-myc transcription may be accompanied by alterations in mRNA initiation or processing that generate different protein products.

249. DETECTION OF THE C-MYC ONCOGENE PRODUCT IN COLONIC POLYPS AND CARCINOMAS

Stewart J. Evan G. Watson J. Sikora K
MRC Clinical Oncology Unit. MRC Centre. Cambridge. UK.
Br J Cancer; 53(1):1-6 1986

The c-myc oncogene has been implicated in the processes of normal cell proliferation and differentiation. Elevated levels of c-myc mRNA and its gene product (p62c-myc), have been detected in a variety of solid tumours and cultured cell lines. Its precise role in normal cell function and in neoplastic transformation and progression has yet to be elucidated. We have used a monoclonal antibody, raised by peptide immunization, to determine the distribution by immunoperoxidase staining of the c-myc oncogene product in archival specimens of colonic polyps and carcinomas. Samples from 42 patients with colon carcinoma, 24 with benign polyps and 15 normal colon biopsies were examined. Normal colon revealed maximum staining in the mid-zone of the crypts, corresponding to the zone of differentiation and maturation. The staining was predominantly cytoplasmic. Adenomatous polyps revealed the most intense pattern of staining in areas of dysplastic change. Colonic tumours showed a wide range of staining. Well differentiated tumours contained more cytoplasmic p62c-myc than poorly differentiated tumours. These findings suggest that the c-myc oncogene product may play an important role in the evolution of colonic neoplasia.

250. TRANSLATION OF VIROGENES AND ONCOGENES IN HUMAN LEUKEMIAS AND LYMPHOMAS AND TRANSMISSION OF CODING DNA (MEETING ABSTRACT)

Toth FD. Vaczi L. Szabo B. Kiss J. Kiss A. Rak K. Kovacs I. Kiss C
Inst. Microbiol., Med. Univ. Sch., 4012 Debrecen, Hungary
XV Meeting of the European Tumour Virus Group. September 19-24. 1984. Urbino. Italy. p. 7. 1984.

We have examined the translational expression of antigens related to p30 polypeptides of baboon endogenous virus (BaEV) and gibbon ape leukemia virus (GaLV) and proteins encoded by myc and src oncogenes by immunofluorescence in human leukemia and lymphoma cells. Most samples irrespective of cell type expressed BaEV p30-like antigen. In contrast expression of antigen related to GaLV p30 was detected in acute myeloid leukemia, blastoid phase of chronic granulocytic leukemia and in B- and O-cell ly mphoid malignancies. Translational expression of myc gene showed lack of specificity. Expression of src was relatively low in most samples, with markedly elevated levels in a few leukemias and lymphoma samples. Transfection of NIH 3T3 cells was performed by the calcium phosphate precipitation technique. Transformed foci expressed both myc and src-specific proteins and very frequently a GaLV p30-like antigen, too.

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251. IDENTIFICATION OF THE C-MYC ONCOGENE PRODUCT IN NORMAL AND MALIGNANT B CELLS

Giallongo A. Appella E. Ricciardi R. Rotera G. Croce C.M
Wistar Institute. Philadelphia. Pennsylvania.
Science: 222(4622):430-2 1983

Antiserum to a synthetic peptide corresponding to the carboxyl-terminus of the human c-myc protein immunoprecipitated a 48,000-dalton protein from a number of normal and malignant human and mouse cells. The size of the protein is consistent with the potential coding region predicted from the c-myc nucleotide sequence, and is the same for malignant cells carrying either a rearranged or an unrearranged c-myc oncogene. Because c-myc transcripts are expressed at higher levels in malignant than in normal B cells, it appears that an increased level of the c-myc protein rather than a change in the gene product is the relevant factor in determining transformation.

252. REARRANGEMENT AND ABNORMAL EXPRESSION OF HUMAN C-MYC IN ACUTE LYMPHOCYTIC LEUKEMIA

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Dept. of Hematology. Istituto Superiore di Sanita. Italy
Genetic and Phenotypic Markers of Tumors. Aaronson SA. Frati L. Verna R. eds. New York. Plenum Press. p. 311-21. 1984.

A case of acute lymphocytic leukemia (ALL), L3 type, with 8;14 chromosome translocation, whereby the c-myc is rearranged into the IgH locus, is reported. Two different c-myc transcripts were detectable at high levels in primary leukemic cells. Both virtually disappeared in the remission phase. These data extend and enhance the significance of the translocation-mediated c-myc activation in the genesis of B-cell neoplasias. The breakpoint of the translocation has been localized within the first intron of the c-myc gene, thus leaving the first untranslated exon on chromosome 8q- and rearranging the whole protein-coding region in the IgH locus on chromosome 14q+. Northern blot analysis revealed high levels of two different c-myc transcripts originated from the translocated gene; these high levels were not detected in WBC in remission phase. For the first time, rearrangement and abnormal expression of a cellular onc gene (c-myc) was demonstrated in primary cells from an ALL patient. (20 Refs)

253. DETECTION OF THE C-MYC ONCOGENE PRODUCT IN TESTICULAR CANCER

Sikora K. Evan G. Stewart J. Watson JV
Ludwig Institute for Cancer Research. Cambridge. UK.
Br J Cancer: 52(2):171-6 1985

A set of monoclonal antibodies was constructed by immunising mice with peptide fragments of the c-myc oncogene product. One such antibody, Myc 1-6E10 was shown to bind to a 62,000 dalton protein identifiable with the c-myc product (p62c-myc). The antigen recognised was not destroyed by paraffin wax embedding. Myc 1-6E10 was used to characterise the distribution of p62c-myc in archival testicular tumour material. Normal testes expressed only small amounts of p62c-myc. Seminomas showed increased nuclear and cytoplasmic staining. Undifferentiated teratoma showed little activity, whereas p62c-myc was abundant in the nuclei of differentiating epithelial structures, yolk sacs and embryoid bodies. Only small amounts of p62c-myc were seen in the tumours of 5 patients who subsequently died from their disease.

254. AMPLIFICATION AND ENHANCED EXPRESSION OF THE C-MYC ONCOGENE IN MOUSE SEWA TUMOUR CELLS

Schwab M. Ramsay G. Alitalo K. Varmus HE. Bishop JM. Martinsson T. Levan G. Levan A
G. W. Hooper Foundation. University of California. School of Medicine. San Francisco 94143.
Nature: 315(6017):345-7 1985

SEWA tumour cells are derived from an osteosarcoma induced in an A.SW mouse by infection with polyoma virus. Cytogenetic analyses have revealed three different characteristic chromosomal abnormalities diagnostic for the presence of amplified genes: 'double minutes' (DMs), homogeneously staining chromosomal regions (HSRs) and C-bandless chromosomes (CMs; for review see ref. 2). DMs may undergo fluctuation in number depending on the conditions in which the cells grow. Their number usually increases after injection of cells into a mouse and often is reduced to undetectable levels when the cells are explanted back into tissue culture; when the cells are re-introduced into the mouse, they again acquire multiple DMs. We show here that cells of SEWA lines carrying DMs, HSRs or CMs contain amplified copies of the proto-oncogene c-myc and enhanced levels of c-myc messenger RNA and c-myc protein. DMs or CMs are the sites of c-myc amplification in two different SEWA lines.

255. CELL-TYPE-SPECIFIC PATTERN OF MYC PROTOONCOGENE EXPRESSION IN DEVELOPING HUMAN EMBRYOS

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Proc Natl Acad Sci USA: 82(15):5050-4 1985

The expression of viral oncogenes in cells transformed by acutely transforming retroviruses profoundly alters proliferation and differentiation in the target cell, suggesting that the cellular homologues of the viral oncogenes, the protooncogenes, have a role in normal cell proliferation and differentiation. To investigate the possible developmental role of protooncogenes in human embryogenesis, we have determined the spatial distribution of myc gene transcripts in early human embryos by using i n situ hybridization of a labeled myc exon to thin sections. The results indicate a stage- and cell-type-specific regulation of c-myc gene expression in primarily epithelial cells of late first trimester embryos. Furthermore, the data suggest that the linkage between c-myc gene expression and cellular proliferation holds for only a restricted set of embryonic cells.

256. KINETICS OF INHIBITION OF C-MYC GENE EXPRESSION BY 1,25 DIHYDROXY VITAMIN D3 (1,25(OH)2D3) IN RELATION TO THE INITIATION OF MONOCYTIC DIFFERENTIATION OF HL 60 CELLS (MEETING ABSTRACT)

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Fed Proc: 44(6):1841 1985

HL 60 cells are derived from a patient with promyelocytic leukemia, and can be induced to differentiate in culture toward either the granulocyte or the monocyte, depending on the chemical inducer chosen. We have recently developed a system for rapid induction of monocytic differentiation in these cells using a short exposure to 1,25(OH)2D3, which permits an analysis of the events related to the initiation of the differentiation pathway. HL 60 cells grown in suspension were exposed to 10(-7)M 1,25(OH)2D3 for 4 hrs and resuspended in normal medium. At intervals, cell sam-

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ples were removed for determination of non-specific esterase (NSE) activity and the ability to phagocytose RBCs, and for extraction of RNA. The poly-A+ RNA was blotted to nitrocellulose and hybridized to 32P labeled cloned cDNA fragment of the c-myc human gene, pMyc 7.4. The results show that steady state levels of c-myc mRNA begin to fall approximately 8 hrs before the first evidence of phenotypic differentiation, but the duration of c-myc mRNA reduction does not correlate with the degree of differentiation. This suggests a complex relationship between c-myc gene expression and 1,25(OH)2D3-induced differentiation of HL 60 cells.

257. MOLECULAR CLONING AND REGULATED EXPRESSION OF THE HUMAN C-MYC GENE IN ESCHERICHIA COLI AND SACCHAROMYCES CEREVISIAE: COMPARISON OF THE PROTEIN PRODUCTS

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Proc Natl Acad Sci USA: 82(21):7232-6 1985

mRNA from human HL-60 cells was used to prepare a cDNA library, from which two full-length clones that encompass the complete c-myc coding region were isolated. One clone, pM1-11, contains all three exons of human c-myc. The second clone, pM4-10, represents a relatively rare transcript that initiated in the first intron and includes the coding exons 2 and 3. The cDNA insert in pM1-11 was used to express the human c-myc protein in both prokaryotic and eukaryotic cells. Insertion of the coding sequences in exons 2 and 3 into the appropriate expression vectors yielded detectable c-myc protein in Escherichia coli lacking the Lon protease and in Saccharomyces cerevisiae upon induction. The protein produced in E. coli has an apparent size of 60 kDa and appears to be unmodified, as it is identical in size to the protein synthesized in an in vitro system. In contrast, yeast cells synthesize two myc proteins, of 60 kDa and 62 kDa. The difference in apparent molecular mass between the two proteins appears to be due, in part, to phosphorylation. Subcellular fractionation of yeast cells showed that the c-myc phosphoprotein is located predominantly in the nuclear fraction.

258. C-MYC ONCOGENE PROTEIN SYNTHESIS IS INDEPENDENT OF THE CELL CYCLE IN HUMAN AND AVIAN CELLS

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Nature. 314(6009):366-9 1985

Several lines of evidence suggest a role for the myc oncogene in cell proliferation. Most recently, mitogenic stimulation of quiescent lymphoid, fibroblast and epithelial cells has been demonstrated to lead to a sharp increase in c-myc RNA levels. To determine how c-myc expression is linked to the cell proliferative cycle, we have used centrifugal elutriation to enrich for populations of avian and human cells at different stages of the cell cycle. Centrifugal elutriation is a counterflow centrifugation method that separates cells on the basis of volume, a parameter correlating well with progression through the cell cycle. Using myc-specific anti-peptide antibodies, we show here that the synthesis, half-life and modification of c-myc proteins are constant throughout the cell cycle of normal and transformed cells.

259. EVIDENCE FOR P15 CLEAVAGE SITE IN MYC-SPECIFIC PROTEINS OF AVIAN MC29 AND OK10 VIRUSES

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Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague.

J Cell Biochem: 28(4):265-72 1985

Myc-related proteins were precipitated from MC29 virus-transformed cells (PR-2) and from OK10 virus-transformed cells (9C) by anti-gag and anti-myc sera. Immunoprecipitates were cleaved with the avian retroviral protease p15 and the cleavage products analyzed in SDS-PAGE. Cleavage fragments of p110gag-myc (product of MC29 virus) and p58myc (product of OK10 virus) showed the presence of a p15 cleavage site within the myc-specific region. The site is missing in deletion mutants of MC29 virus.

260. PROTEINS ENCODED BY THE HUMAN C-MYC ONCOGENE: DIFFERENTIAL EXPRESSION IN NEOPLASTIC CELLS

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Mol Cell Biol: 4(11):2486-97 1984

To examine myc protein products in the wide variety of human tumor cells having alterations of the c-myc locus, we have prepared an antiserum against a synthetic peptide corresponding to the predicted C-terminal sequence of the human c-myc protein. This antiserum (anti-hu-myc 12C) specifically precipitated two proteins of 64 and 67 kilodaltons in quantities ranging from low levels in normal fibroblasts to 10-fold-higher levels in Epstein-Barr virus-immortalized and Burkitt's lymphoma cell lines, to 20- to 60-fold-higher levels in cell lines having amplified c-myc. The p64 and p67 proteins were found to be highly related by partial V8 proteolytic mapping, and both were demonstrated to be encoded by the c-myc oncogene, using hybrid-selected translation of myc-specific RNA. In addition, the p64 protein was specifically precipitated from cells transfected with a translocated c-myc gene. Both p64 and p67 were found to be nuclear phosphoproteins with extremely short half-lives. In tumor cell lines having alterations at the c-myc locus due to amplification or translocation, we observed a significant change in the expression of p64 relative to p67 when compared with normal or Epstein-Bar virus-immortalized cells.

261. REGULATION OF HEAT SHOCK PROTEIN 70 GENE EXPRESSION BY C-MYC

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Nature: 312(5991):280-2 1984

The myc gene seems to have a causal role in tumour formation in man, mouse and avian systems. The myc gene product has been localized to the nucleus, suggesting that it may be involved in the regulation of gene expression. The level of expression of the mammalian heat shock protein 70 (HSP70) gene is elevated in several tumour cell lines, implying that a cellular function expressed in these tumour lines can stimulate HSP70 production. We report here that the gene product of a rearranged mouse c-myc gene is capable of stimulating expression of chimaeric genes containing a Drosophila hsp70 promoter region and 5'-flanking sequences. This stimulation is dependent on sequences located more than 200 bases 5' of the normal start of hsp70 transcription.

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262. ANTIBODIES TO HUMAN C-MYC ONCOGENE PRODUCT: EVIDENCE OF AN EVOLUTIONARILY CONSERVED PROTEIN INDUCED DURING CELL PROLIFERATION

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Science: 225(4662):687-93 1984

Antisera to a synthetic c-myc peptide and to c-myc antigens synthesized from various portions of the human gene expressed in Escherichia coli were used in order to characterize the protein product of the human c-myc oncogene. Although the deduced molecular weight of the human c-myc protein is 49,000, these antisera precipitate a protein from human cells that migrates in sodium dodecyl sulfate-polyacrylamide gel as if its molecular weight were 65,000. In addition, the mouse c-myc protein, whether synthesized in cells or in a cell-free system directed by pure, synthetic messenger RNA, has analogous properties and is immunoprecipitated by the antiserum to the human c-myc protein. Similar proteins are immunoprecipitated from monkey, rat, hamster, and frog cells, suggesting evolutionary conservation of antigenic structure of the c-myc protein among vertebrates. In addition, and in a manner consistent with the behavior of its messenger RNA, the immunoprecipitable c-myc protein is sharply induced by the action of mitogens on resting human T cells.

263. NUCLEOTIDE SEQUENCE OF THE HUMAN N-MYC GENE

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Proc Natl Acad Sci USA: 83(6):1772-6 1986

Human neuroblastomas frequently display amplification and augmented expression of a gene known as N-myc because of its similarity to the protooncogene c-myc. It has therefore been proposed that N-myc is itself a protooncogene, and subsequent tests have shown that N-myc and c-myc have similar biological activities in cell culture. We have now detailed the kinship between N-myc and c-myc by determining the nucleotide sequence of human N-myc and deducing the amino acid sequence of the protein encoded by the gene. The topography of N-myc is strikingly similar to that of c-myc: both genes contain three exons of similar lengths; the coding elements of both genes are located in the second and third exons; and both genes have unusually long 5' untranslated regions in their mRNAs, with features that raise the possibility that expression of the genes may be subject to similar controls of translation. The resemblance between the proteins encoded by N-myc and c-myc sustains previous suspicions that the genes encode related functions.

264. NUCLEOTIDE SEQUENCE COMPARISON OF NORMAL AND TRANSLOCATED MURINE C-MYC GENES

Stanton LW. Fahrlander PD. Tesser PM. Marcu KB
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Nature: 310(5976):423-5 1984

Plasmacytoid tumours in mice and Burkitt's lymphomas in humans display characteristic chromosomal translocations involving the c-myc proto-oncogene. Models to explain quantitative changes in c-myc expression have been proposed based on the loss of normal promoters as a result of translocation. However, alternative explanations, such as somatic mutation are needed to explain altered c-myc expression in the absence of gene breakage. We present here the nucleotide sequence of the normal murine c-myc gene. Comparison of this sequence with that of a translocated c-myc gene from a murine plasmacytoma reveals complete identity of coding sequence. One nucleotide difference was found in the non-coding first exon. This shows that qualitative changes of the c-myc gene product are not required for oncogenesis in murine plasmacytomas. In contrast, mutations are found in coding and non-coding regions of translocated c-myc genes from Burkitt's lymphomas, suggesting that the mechanisms by which c-myc is activated in plasmacytomas and Burkitt's lymphomas are different.

265. NUCLEOTIDE SEQUENCE OF THE HUMAN C-MYC LOCUS: PROVOCATIVE OPEN READING FRAME WITHIN THE FIRST EXON

Gazin C. Dupont de Dinechin S. Hampe A. Masson JM. Martin P. Stehelin D. Galibert F
Laboratoire d'Hematologie Experimentale, Hopital Saint-Louis, Paris, France.
EMBO J: 3(2):383-7 1984

The nucleotide sequence of a HindIII-EcoRI DNA fragment, 8 kbp long, of a lambda recombinant containing the whole human c-myc gene has been deduced by the method of Maxam and Gilbert. This fragment encodes the complex c-myc locus and the sequence provides information relative to the 2.7 kb long c-myc transcript. It appears that although exons 2 and 3 would code for a 48-K protein homologous to the myc domain of the viral p110 gag-myc protein, the first exon, which has a large open reading frame ending with a stop codon just upstream from the donor splice site, could code on its own for a 20-K protein. Speculations about the role of that putative protein on the regulation of the expression of exons 2 and 3 are made.

266. SELECTIVE REDUCTION OF C-MYC MRNA IN DAUDI CELLS BY HUMAN BETA INTERFERON

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Proc Natl Acad Sci USA: 81(6):1747-50 1984

Under normal growth conditions, the human lymphoblastoid cell line Daudi expresses high levels of c-myc mRNA. These cells are also sensitive to growth inhibition by interferons. We have compared the levels of mRNA for the c-myc in untreated and human beta interferon (IFN-beta)-treated Daudi cells by RNA dot-blot and blot-hybridization analysis methods. Using a synthetic oligonucleotide complementary to the human c-myc mRNA as the probe, we detected a more than 75% reduction in the c-myc hybridizable poly(A)+ RNA in the IFN-beta-treated cells. This reduction in the c-myc mRNA appears to be selective because the level of actin mRNA is not significantly affected by the IFN-beta treatment. In addition, neither in vitro translation of mRNA extracted from IFN-beta-treated cells nor in vivo synthesis of cellular proteins in IFN-beta-treated cells are quantitatively affected. We surmise that the selective reduction in the amount of c-myc mRNA in IFN-beta-treated Daudi cells may be related to the IFN-induced inhibition of the Daudi tumor cell growth.

267. STRUCTURE AND EXPRESSION OF THE MURINE N-MYC GENE

DePinho RA. Legouy E. Feldman LB. Kohl NE. Yancopoulos GD. Alt FW
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Proc Natl Acad Sci USA: 83(6):1827-31 1986

We have demonstrated that the entire murine N-myc gene and the sequences necessary for its expression in human

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neuroblastoma cells are contained within a 7.4-kilobase murine genomic clone. The complete nucleotide sequence of this gene reveals a number of striking similarities and differences when compared to the related c-myc gene including the following: (i) each gene contains three exons of which the first encodes a long 5'-untranslated leader sequence; (ii) the coding regions of the N- and c-myc genes share regions of substantial nucleic acid homology, the putative N-myc protein shares substantial homology with the c-myc protein; (iii) as with c-myc, extensive nucleotide sequence homology exists between the untranslated regions of the human and murine N-myc gene transcripts; however, the N-myc and c-myc untranslated regions are totally divergent; (iv) the N-myc transcriptional promoter differs from that of c-myc and is more related to the promoter of the simian virus 40. We discuss these findings in the context of previously defined similarities and differences in the potential functional and regulatory aspects of these two myc-family members.

268. NUCLEOTIDE SEQUENCE OF THE 3' EXON OF THE HUMAN N-MYC GENE

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Memorial Sloan-Kettering Cancer Center, Graduate Program in Molecular Biology and Virology, New York, NY 10021.
Nucleic Acids Res; 13(7):2545-58 1985

We have analyzed a 3.8 kb Eco RI fragment of genomic DNA obtained from the amplified N-myc gene of human neuroblastoma cell line BE(2)-C. This fragment contains an exon with an open reading frame encoding approximately 170 amino acids of the carboxy-terminal end of the putative N-myc protein. Comparison of the inferred amino acid sequence of this peptide with that of the 3' domain of the human c-myc protein shows that locally conserved but dispersed regions of homology exist throughout the lengths of these peptides, while hydropathy plots indicate that the physical properties implied by their primary sequences are strikingly similar. Based upon these and other considerations, it is suggested that the 3' domains of c-myc and N-myc may potentially share related functions.

269. A SIMULTANEOUS FLOW CYTOMETRIC ASSAY FOR C-MYC ONCOPROTEIN AND DNA IN NUCLEI FROM PARAFFIN EMBEDDED MATERIAL

Watson JV, Sikora K, Evan GI
MRC Clinical Oncology Unit, Medical School, Cambridge, U.K.
J Immunol Methods; 83(1):179-92 1985

A simultaneous flow cytometric assay for the c-myc oncoprotein and DNA in nuclei extracted from archival paraffin wax embedded clinical biopsies is presented. The nuclei were extracted by pepsin digestion after dewaxing 20 micron sections. The c-myc oncoprotein was probed with a mouse monoclonal antibody. This was raised against a synthetic peptide corresponding to a hydrophilic region of the protein predicted from the amino acid sequence. The technique is illustrated with biopsies from patients with testicular cancer and with benign and malignant neoplasms of the colon.

270. ACTIVATION OF THE C-MYC ONCOGENE (MEETING ABSTRACT)

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International Conference on Mechanisms of DNA Damage and Repair: Implications for Carcinogenesis and Risk Assessment. Invited and Short Oral Presentations, June 2-7, 1985, Gaithersburg, Maryland, 1985.

The c-myc oncogene has been implicated in the genesis of a wide range of cancers, including B and T cell lymphomas, malignant variants of small cell lung carcinomas, a promyelocytic leukemia and a neuroendocrine tumor. The mechanisms of activation involve chromosomal translocation, gene amplification and proviral integration. The most extensively studied mechanism of c-myc activation is chromosomal translocation, which in mouse plasmacytomas and Burkitt lymphomas involves linkage of c-myc to immunoglobulin sequences. After translocation, the c-myc gene is expressed at high levels, similar to those found in mitogen-stimulated normal B cells. Two different models have been proposed to account for the transcriptional activation of c-myc. One suggests that linkage of c-myc to the immunoglobulin domain induces increased expression through a positive influence. A second model suggests that c-myc is under negative regulatory control and that translocation prevents the interaction of a 'repressor' molecule. Most of the evidence to date points to the positive influence of the immunoglobulin domain on deregulated c-myc expression. In addition to the deregulation of c-myc expression that is associated with the mechanisms above, DNA sequence studies of cloned tumor genes have revealed infrequent mutations, mainly within the first non-translated exon. No increase in transforming activity has been demonstrated for these mutations, which have been described only in association with translocations. Both translocation and amplification alter the regulation of c-myc protein levels, suggesting that quantitative rather than qualitative changes in c-myc expression are most likely to influence normal cells to grow aberrantly. However, mutations that activated the transforming potential of the c-myc protein, without altering gene regulation, would not have been detected with the presently available assays. The role of c-myc activation in cell transformation remains controversial. Land et al (Nature 304:596-602, 1983) suggested that c-myc contributes to the 'immortalization' of primary cells, while activated ras genes are required for 'transformation.' My laboratory (Keath et al, Cell 39:339-348, 1984) has demonstrated that activated c-myc genes can induce tumorigenicity in previously immortalized fibroblast lines. Moreover, subsequent studies (Spandidos and Wilke, Nature 310:469-475, 1984) have shown that low level expression of the mutant ras protein leads to immortalization of primary cells rather than transformation. We have recently found that transfection of activated myc genes into ras-immortalized cells does not induce foci, but that the cells do become tumorigenic. High levels of c-myc expression alone can induce a similar phenotype (unpublished observations). Thus, depending on the dosage, a particular activated oncogene can either partially or fully transform primary cells. Additional studies will be required to resolve the contribution of c-myc oncogene activation of the malignant phenotype of tumor cells in which c-myc has been implicated. (3 Refs)

271. ABROGATION OF IL-3 AND IL-2 DEPENDENCE BY RECOMBINANT MURINE RETROVIRUSES EXPRESSING V-MYC ONCOGENES

Rapp UR, Cleveland JL, Brightman K, Scott A, Ihle JN
Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701.
Nature; 317(6036):434-8 1985

Several oncogenes are thought to cause transformation by affecting the signal transmission pathway of growth factors. One example is the induction of c-myc, the cellular homologue of the avian transforming oncogene v-myc, by platelet-derived growth factor (PDGF) among a set of genes associated with competence induction in fibroblasts. Another of the competence genes, r-fos, has been shown to be related to v-fos, the transforming gene of the FBJ sarcoma virus. In addition, PDGF induces c-fos, the cellular homologue of v-fos. The importance of c-myc induction is suggested by the ob-

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servation that c-myc, under the control of a glucocorticoid regulator, can partially relieve the requirement of fibroblasts for PDGF. We have examined the effects of oncogenes on haematopoietic/lymphoid cell differentiation, immortalization and factor dependence for growth. Here we report the effects of recombinant murine retroviruses capable of expressing the avian v-myc. With interleukin-3 (IL-3)- or interleukin-2 (IL-2)-dependent cells, the viruses abrogated the requirement for growth factors and suppressed c-myc expression.

272. THE LONG TERMINAL REPEAT OF THE INTRACISTERNAL A PARTICLE AS A TARGET FOR TRANSACTIVATION BY ONCOGENE PRODUCTS

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J Virol; 57(3):998-1003 1986

It has been shown recently that the c-mos oncogene becomes activated in myeloma XRPC-24 via insertion of an intracisternal A particle (IAP) long terminal repeat (LTR). The inserted LTR serves as a promoter from which transcription of the 3' rearranged c-mos initiates. The insertion is in a head-to-head orientation such that the transcriptional orientations of the IAP and the 3' rearranged c-mos are opposite. It has already been shown that this IAP LTR has two promoters, one transcribing the IAP genome and the other transcribing the rearranged c-mos. Since the IAP genomes are actively transcribed in mouse myelomas but not in normal cells, it was interesting to test whether transcriptional act ivation of the IAP occurs in the presence of active oncogene products, especially nuclear ones. The 5' LTR of the IAP inserted in myeloma XRPC-24 was chosen as a convenient model to test the effect of viral and cellular oncogene products. These included simian virus 40 (SV40) large-T antigen, the adenovirus early 1A (E1A) gene product, the myc gene product, and p53. The LTR was coupled to the bacterial gene coding for chloramphenicol acetyltransferase (CAT) in two orientations, and the levels of CAT directed by the LTR promoters were assayed in either the presence or the absence of the oncogene products. The levels of CAT directed by the 5' LTR promoter transcribing the IAP were significantly elevated in the presence of SV40 large-T antigen, the adenovirus E1A and myc gene products, and p53. The promoter transcribing the rearranged c-mos was transactivated by SV40 large-T antigen and the adenovirus E1A gene product. The results indicate that oncogene products may have an important role in turning on promoters of other genes. The IAP LTR may serve as a useful model for studying the effect of various gene products on promoters which are known to be activated in the malignant state.

273. PROPERTIES OF THE MYC-GENE PRODUCT: NUCLEAR ASSOCIATION, INHIBITION OF TRANSCRIPTION AND ACTIVATION IN STIMULATED LYMPHOCYTES

Moelling K. Benter T. Bunte T. Pfaff E. Deppert W. Egly J.M. Miyamoto NB

Curr Top Microbiol Immunol. 113:198-207 1984

Keywords (MeSH): Animal; Antibodies; Antigen-Antibody Complex; Base Sequence; Cell Line; Cell Nucleus/*metabolism; *Cell Transformation, Neoplastic; Fluorescent Antibody Technic; *Genes, Viral; Hela Cells/analysis; Human; Lymphocytes/*physiology; Neoplasm Proteins/*genetics; *Oncogenes; Quail; Retroviridae/*genetics; *Transcription, Genetic.

274. IDENTIFICATION OF THE HUMAN CELLULAR MYC GENE PRODUCT BY ANTIBODY AGAINST THE BACTERIALLY EXPRESSED PROTEIN

Benter T. Beimling P. Pfaff E. Sander T. Heimann B. Moelling K

Max-Planck-Institut fur Molekulare Genetik, Berlin, FRG.

Hamatol Bluttransfus; 29:266-8 1985

Keywords (MeSH): Antibodies; Cloning, Molecular; Coliphages/genetics; Escherichia Coli/genetics; Fluorescent Antibody Technic; Hela Cells/cytology; Human; Molecular Weight; Neoplasm Proteins/*genetics/isolation and purification; *Oncogenes; Plasmids.

275. THE ROLE OF C-MYC IN THE PROLIFERATION OF NORMAL AND NEOPLASTIC CELLS (50 Refs)

Kelly K. Siebenlist U

Immunology Branch, National Cancer Institute, Bethesda, Maryland 20205.

J Clin Immunol 5(2):65-77 1985

Keywords (MeSH): Animal; Burkitt's Lymphoma/familial and genetic/*pathology; Cell Cycle; *Cell Division; Cell Transformation, Neoplastic; Cell Transformation, Viral; Gene Expression Regulation; Growth Substances/physiology; Human; *Oncogenes; Review; Transcription, Genetic; Translocation (Genetics).

276. MYC-ENCODED PROTEINS OF CHICKENS AND MEN

Eisenman RN. Hann SR

Curr Top Microbiol Immunol; 113:192-7 1984

Keywords (MeSH): Animal; Burkitt's Lymphoma; Cell Line; Chickens; Comparative Study; Hela Cells/analysis; Human; Molecular Weight; Neoplasm Proteins/*genetics/isolation and purification; *Oncogenes; Species Specificity.

277. DOES THE LARGE T PROTEIN OF POLYOMA VIRUS REGULATE THE EXPRESSION OF THE CELLULAR MYC GENE?

Glaichenhaus N. Galup C. Mougneau E. Cuzin F

Curr Top Microbiol Immunol; 113:20-5 1984

Keywords (MeSH): Animal; Base Sequence; Cell Line; *Cell Transformation, Neoplastic; Human; *Oncogenes; Phenotype; Polyomaviruses/*genetics; Protein Kinases/*metabolism; Rats; Viral Proteins/*metabolism.

3.

p53 protein product

278. HUMAN P53 GENE LOCALIZED TO SHORT ARM OF CHROMOSOME 17

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Nature; 319(6056):783-4 1986

The p53 gene codes for a nuclear protein that has an important role in normal cellular replication. The concentration of p53 protein is frequently elevated in transformed cells. Transfection studies show that the p53 gene, in collaboration with the activated ras oncogene, can transform cells. Chromosomal localization may provide a better understanding of the relationship of p53 to other human cellular genes and of its possible role in malignancies associated with specific chromosomal rearrangements. A recent study mapped the human p53 gene to the long arm of chromosome 17 (17q21-q22) using in situ chromosomal hybridization. Here, by Southern filter hybridization of DNAs from human-rodent hybrids, we have localized the p53 gene to the short arm of human chromosome 17.

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279. TRANSLOCATION OF THE P53 GENE IN T(15;17) IN ACUTE PROMYELOCYTIC LEUKAEMIA

Le Beau MM. Westbrook CA. Diaz MO. Rowley JD. Oren M
Department of Medicine. University of Chicago. Illinois 60637.
Nature: 316(6031):826-8 1985

Recent studies have demonstrated that the cellular tumour antigen p53 (ref. 1) can complement activated ras genes in the transformation of rat fibroblasts, suggesting that the gene encoding p53 may act as an oncogene. Here, by using in situ chromosomal hybridization, we have mapped the p53 gene to human chromosome 17, at bands 17q21-q22, the region containing one of the breakpoints in the translocation t(15;17) (q22;q21) associated with acute promyelocytic leukaemia (APL). Hybridization of p53 and erb-A (17q11-q12) probes to malignant cells from three APL patients indicated that the p53 gene is translocated to chromosome 15 (15q+), whereas erb-A remains on chromosome 17. Analysis of variant translocations demonstrates that the 15q+ chromosome contains the conserved junction, suggesting a role for p53 in the pathogenesis of APL. However, rearrangements of the p53 gene were not detected on Southern blotting of DNA from leukaemic cells of four APL patients with t(15;17).

280. ANALYSIS OF HUMAN P53 PROTEINS AND MRNA LEVELS IN NORMAL AND TRANSFORMED CELLS

Matlashewski G. Banks L. Pim D. Crawford L
Laboratory of Molecular Virology. Imperial Cancer Research Fund. London. England.
Eur J Biochem: 154(3):665 72 1986

p53 mRNA and proteins were examined in a variety of human transformed cells and in normal human foreskin fibroblast cells. Both the steady-state and translatable levels of p53 mRNA were the same in normal and transformed human cells. In vitro synthesized p53, programmed by mRNA from normal and transformed human cells, revealed that there was heterogeneity in the primary structure of p53 from these cells. Pulse labeling of cells and immunoprecipitation analysis with a panel of human reactive anti-p53 antibodies demonstrated that the types of p53 synthesized in vitro corresponded to the types made in vivo from SV80 and COLO 320 cells. No p53 was detectable by similar pulse-labeling analysis of HeLa and normal foreskin fibroblast cells. Since it was necessary to use anti-p53 sera from cancer patients to carry out much of the immunoprecipitation analysis in this study we therefore further characterised these sera to determine if they reacted with one or more than one epitope. p53-beta-galactosidase fusion proteins were synthesized in Escherichia coli and used to analyse the anti-p53 antibodies produced by cancer patients. We demonstrate that the antisera contain antibodies directed against epitopes in both the N-terminal and C-terminal regions of the p53 molecule.

281. COOPERATION BETWEEN GENE ENCODING P53 TUMOUR ANTIGEN AND RAS IN CELLULAR TRANSFORMATION

Parada LF. Land H. Weinberg RA. Wolf D. Rotter V
Whitehead Institute for Biomedical Research. Center for Cancer Research. Cambridge. Massachusetts.
Nature: 312(5995):649-51 1984

The protein p53 is highly expressed in a large variety of transformed cell types originating from diverse species. These include cells transformed by Simian virus 40 (SV40), adenovirus and Abelson virus, as well as a variety of chemically transformed cells. Substantial amounts of p53 are also present in certain non-transformed cells, for example, some embryonic tissues. The protein may be localized in different cellular compartments in normal and transformed cells. The strong correlation between tumorigenicity and high levels of p53 suggests an important role of p53 in tumorigenesis. We report here experiments in which we have co-transfected the murine cellular gene encoding for p53 with a ras gene into primary rat embryo fibroblasts. Our results indicate that the p53-encoding gene can play a causal role in the conversion of normal fibroblasts into tumorigenic cells.

282. CELLULAR IMMORTALIZATION BY A CDNA CLONE ENCODING THE TRANSFORMATION-ASSOCIATED PHOSPHOPROTEIN P53

Jenkins JR. Rudge K. Currie GA
Marie Curie Memorial Foundation. Research Institute. Surrey. UK.
Nature: 312(5995):651-4 1984

Malignant transformation of primary cells requires at least two distinct and characteristic alterations in cellular behaviour. The first, cellular immortality, can be induced by chemical carcinogens or by cloned oncogenes such as polyoma large T (ref. 4), adenovirus early region 1A (E1A) or the oncogene from avian (MC29) myelocytomatosis virus, v-myc. Cells whose in vitro life-span has been extended by these procedures can be fully transformed by transfection with oncogenes belonging to a different complementation group, including genes of the ras family, adenovirus E1b and polyoma virus middle T (refs 4, 5). The unstable cellular phosphoprotein p53 is frequently present at elevated levels in transformed cells and is stabilized by the formation of complexes with simian virus 40 (SV40) large T or adenovirus E1b 57K protein. Although several reports have associated p53 with cell proliferation, its role remains obscure. We have cloned complementary DNA sequences encoding murine p53 and report here that transfection of p53 expression constructs into cells of finite lifespan in vitro results in cellular immortality and susceptibility to transformation by a ras oncogene.

283. PARTICIPATION OF P53 CELLULAR TUMOUR ANTIGEN IN TRANSFORMATION OF NORMAL EMBRYONIC CELLS

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Department of Chemical Immunology. Weizmann Institute of Science. Rehovot. Israel.
Nature: 312(5995):646-9 1984

The cellular tumour antigen p53 is found at elevated levels in a wide variety of transformed cells (for reviews see refs 1, 2). Very little is yet known about the precise relationship of p53 to malignant transformation. Although the increase in p53 levels could be a secondary by-product of the transformed state, it is equally possible that p53 is actively involved in altering cellular growth properties, especially as it has been implicated in the regulation of normal cell proliferation. We sought to test whether p53 could behave in a manner similar to known genes in a biological test system, and we demonstrate here that p53 can cooperate with the activated Ha-ras oncogene to transform normal embryonic cells. The resultant foci contain cells of a markedly altered morphology which produce high levels of p53. Cell lines established from such foci elicit tumours in syngeneic animals.

284. ANALYSIS OF THE GENE CODING FOR THE MURINE CELLULAR TUMOUR ANTIGEN P53

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EMBO J: 3(9):2179-83 1984

A genomic clone containing the functional gene for the murine p53 cellular tumour antigen was isolated and structurally characterised. The gene contains at least 11 exons and 10 introns, the first intron possessing a length of 6.1 kb. Attempts to determine the exact 5' end of p53 mRNA were inconclusive, probably due to the presence of a remarkable

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stem and loop structure (delta G degrees approximately equal to -56 kcal/mol) in the 5' region of the gene. Suggestive similarities were found to exist between p53 and the protein product of the myc oncogene.

285. RECONSTITUTION OF P53 EXPRESSION IN A NONPRODUCER AB-MULV-TRANSFORMED CELL LINE BY TRANSFECTION OF A FUNCTIONAL P53 GENE

Wolf D. Harris N. Rotter V
Department of Cell Biology. Weizmann Institute of Science. Rehovot. Israel.
Cell; 38(1):119-26 1984

L12 are Ab-MuLV-transformed cells that express the abl p120 oncogene product but lack the cellularly encoded p53. The functional p53 gene in these cells has been inactivated by the insertion of Moloney virus-like sequences into the first p53 intron. Transfection of L12 cells with a functional p53 gene, contained in a 16 kb Eco RI genomic cloned fragment gave rise to L12-derived cell lines with novel p53 sequences of various sizes and copy number. A high percentage of L12-derived clones efficiently transcribed p53 mRNA and synthesized the p53 protein. Whereas injection of L12 parental cells into syngeneic mice caused the development of local tumors that later regressed, L12-derived clones that expressed p53 caused lethal tumors in syngeneic mice, thus behaving similarly to other Ab-MuLV-transformed p53-producer cell lines. These results suggest that the expression of p53 is essential for tumor cells to exhibit a fully transformed phenotype, manifested in lethal tumors in syngeneic mice.

286. THE PRESENCE OF P53 TRANSFORMATION-RELATED PROTEIN IN AB-MULV TRANSFORMED CELLS IS REQUIRED FOR THEIR DEVELOPMENT INTO LETHAL TUMORS IN MICE

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Int J Cancer; 31(3):315-320 1983

A cellular-encoded protein, p53, is synthesized at elevated levels in a wide range of tumor cells. Ab-MuLV-transformed cells expressing both the viral-encoded p120 oncogene and the cellular-encoded p53 display a lethal tumor phenotype in syngeneic mice. L12 is an exceptional Ab-MuLV-transformed cell line that expresses the p120 oncogene and lacks the p53 cellular protein. Injection of L12 cells into syngeneic mice is followed by the development of local tumors that are subsequently rejected. Prolonged treatment of L12 cells with TPA, a tumor cell promoter, gave rise to L12T cells that synthesize the p53 protein and exhibit a lethal tumor phenotype. Comparison of one-dimensional proteolytic partial peptide map of p53 obtained from L12T to that obtained from other Ab-MuLV-transformed cell lines confirmed their identity. These results suggest a correlation between the cellular expression of p53 in Ab-MuLV-transformed cells and their capacity to develop into lethal tumors in syngeneic mice (Author abstract) (37 Refs)

287. CLONING AND EXPRESSION ANALYSIS OF FULL LENGTH MOUSE CDNA SEQUENCES ENCODING THE TRANSFORMATION ASSOCIATED PROTEIN P53

Jenkins JR. Rudge K. Redmond S. Wade-Evans A
Marie Curie Memorial Foundation Research Institute. Oxted. Surrey. UK.
Nucleic Acids Res; 12(14):5609-26 1984

We have cloned and sequenced overlapping cDNA fragments which together encode the entire mouse protein p53. Using these cDNA's we have reconstructed the full length coding region for the protein, and have analysed its coding potential by expression in vitro, both as a full length sequence and as a subfragment contained in a fusion protein. The predicted amino acid sequence contains no obvious homologies to any known oncogenes but includes a possible tyrosine kinase acceptor site.

4.

fos protein product

288. C-FOS PROTEIN CAN INDUCE CELLULAR TRANSFORMATION: A NOVEL MECHANISM OF ACTIVATION OF A CELLULAR ONCOGENE

Miller AD. Curran T. Verma IM
Molecular Biology and Virology Laboratory. Salk Institute. San Diego. California.
Cell; 36(1):51-60 1984

The FBJ murine osteosarcoma virus (FBJ-MuSV) induces tumors in vivo and transformation in vitro. Transformation is due to the expression of a single viral protein (p55v-fos) which is encoded by sequences derived from mouse genetic material. The homologous cellular gene (c-fos) does not transform cultured cells after introduction by transfection. We show that even though the c-fos protein is completely different from the v-fos protein at its C terminus, it is capable of transforming cultured fibroblasts. However, activation of the transforming potential of the c-fos gene requires two manipulations - a transcriptional enhancer sequence must be linked to the gene and an interaction at the 3' end of the gene, which inhibits transformation, must be disrupted. Our studies show that normal cellular protein can induce transformation when expressed in an inappropriate cell type.

289. INDUCTION OF C-FOS DURING MYELOMONOCYTIC DIFFERENTIATION AND MACROPHAGE PROLIFERATION

Muller R. Curran T. Muller D. Guilbert L
European Molecular Biology Laboratory. Heidelberg. FRG.
Nature; 314(6011):546-8 1985

Previous studies have suggested a role for c-fos in cellular differentiation in fetal membranes, haematopoietic cells and teratocarcinoma stem cells. In other cell types, such as fibroblasts, c-fos expression is normally very low, but is rapidly induced by peptide growth factors, implicating c-fos in growth control mechanisms. Here, we show that the TPA (12-O-tetradecanoylphorbol-13-acetate)-induced macrophage-like differentiation of HL60 human promyelocytic precursor cells is accompanied by the induction of both c-fos mRNA and protein within 15 min after treatment, suggesting a functional role for c-fos in this differentiation system. In quiescent terminally differentiated macrophages, expr ession of c-fos is inducible by the macrophage-specific growth factor colony-stimulating factor-1 (CSF-1). The kinetics of c-fos induction, however, are entirely different from those in growth factor-stimulated fibroblasts, supporting the view that the c-fos gene product may serve different functions in different cell types.

290. RAPID INDUCTION OF THE EXPRESSION OF PROTO-ONCOGENE FOS DURING HUMAN MONOCYTIC DIFFERENTIATION

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Molecular Biology and Virology Laboratory. Salk Institute. San Diego. California.
Cell; 40(1):209-17 1985

The differentiation into macrophages of the monocytic cell line U-937 or the monomyelocytic cell line HL-60 induced by phorbol esters is accompanied by a rapid induction of the expression of the proto-oncogene c-fos. Maximal levels of c-fos gene transcripts accumulate within 20-30 min following in-

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duction. By 2 hr, the c-fos-specific mRNA level drops 5 to 10 fold below that detected at 30 min after induction. The concentration of c-fos mRNA remains unchanged for the next 5 days, at which time over 99% of the viable precursor cells are converted to adherent macrophages. c-fos protein can be detected within 20 min of induction, reaching maximal levels by 40-60 min. Little or no c-fos protein is detected by 120-150 min after induction, even though c-fos mRNA remains detectable. Presence of cycloheximide leads to superinduction of c-fos mRNA transcripts. No c-fos gene transcripts are detected when the HL-60 cell line is induced with Me2SO to differentiate to granulocytes. A role for the c-fos protein is envisaged in the monocyte differentiation pathway.

291. SUPERINDUCTION OF C-FOS BY NERVE GROWTH FACTOR IN THE PRESENCE OF PERIPHERALLY ACTIVE BENZODIAZEPINES

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Science: 229(4719):1265-8 1985

Alterations in proto-oncogene expression after stimulation of rat pheochromocytoma (PC12) cells by nerve growth factor (NGF) have been investigated. A specific stimulation of c-fos messenger RNA and protein was detected 30 minutes after treatment. This induction was enhanced more than 100-fold in the presence of peripherally active benzodiazepines. The effect was specific as very little change was observed in the levels of c-rasHa, c-rasKi, c-myc, and N-myc messenger RNA's. Under the conditions used here, NGF treatment ultimately results in neurite outgrowth, with a reduction or cessation of cell division. Thus, stimulation of the c-fos gene in this system appeared to be associated with differentiation and not with cellular proliferation. The effect of benzodiazepines was stereospecific and represents a novel action of these compounds at the level of gene expression.

292. PLATELET-DERIVED GROWTH FACTOR INDUCES RAPID BUT TRANSIENT EXPRESSION OF THE C-FOS GENE AND PROTEIN

Kruijer W. Cooper JA. Hunter T. Verma IM
Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California.
Nature: 312(5996):711-6 1984

Exposure of quiescent mouse fibroblasts to platelet-derived growth factor induces mRNA from the c-fos proto-oncogene within 10 min followed by synthesis of nuclear c-fos proteins. These are amongst the earliest described nuclear events that follow a mitogenic stimulus.

293. DIFFERENTIAL EXPRESSION OF C-FOS IN HEMATOPOIETIC CELLS: CORRELATION WITH DIFFERENTIATION OF MONOMYELOCYTIC CELLS IN VITRO

Muller R. Muller D. Guilbert L
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EMBO J: 3(8):1887-90 1984

Among various neonatal mouse tissues, elevated levels of c-fos transcripts have been detected in crude preparations of bone. Here, we show that c-fos expression originates in the bone marrow, and to a lesser extent in adherent cells (macrophages). High levels of c-fos expression were also detected in primary cultures of differentiated bone marrow-derived macrophages, but not in cell lines resembling immature hematopoietic cells. However, when the human promyelocytic cell line HL60 was induced in vitro to differentiate into macrophages, c-fos expression was readily detectable. These findings suggest that c-fos expression in mononuclear phagocytic cells is restricted to late stages of differentiation. This pattern of c-fos expression is in marked contrast to those of the c-myb and c-myc genes which are transcriptionally active predominantly in precursor cells. In the amnion, where c-fos expression increases to high levels at late stages of gestation, synthesis of c-fos protein occurs in cells that do not resemble macrophages. It thus appears that c-fos expression in several distinct cell types is correlated with differentiation processes.

294. DIFFERENTIATION OF F9 TERATOCARCINOMA STEM CELLS AFTER TRANSFER OF C-FOS PROTO-ONCOGENES

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European Molecular Biology Laboratory, Heidelberg, FRG.
Nature: 311(5985):438-42 1984

Transfer of mouse or human c-fos proto-oncogenes into F9 teratocarcinoma stem cells results in expression of c-fos mRNA and protein. This is accompanied by the appearance of morphologically altered cells which express several specific markers characteristic of differentiated cells, suggesting that c-fos plays a role in cellular differentiation.

295. DIFFERENTIAL EXPRESSION OF CELLULAR ONCOGENES DURING PRE- AND POSTNATAL DEVELOPMENT OF THE MOUSE

Muller R. Slamon DJ. Tremblay JM. Cline MJ. Verma IM
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Nature: 299(5884):640-644 1982

Differential transcriptional activities of cellular homologs (c-onc) of viral oncogenes (v-onc) were studied in embryonic and postnatal mice. The cellular homolog (c-fos) of the viral transforming gen e product (v-fos) of FBJ murine osteosarcoma virus was found in 6-, 7-, 8-, and 9-day conceptuses and extraembryonic membranes (EEM). C-fos expression was 10-fold lower in later embryos without EEM. In 10-day embryos c-fos expression was higher in the placenta than in the embryo or EEM. High c-fos expression in 18-day placenta and the absence in 12-day EEM suggest that c-fos is expressed at an early stage in cells that give rise to the placenta in later stages. In postnatal mice c-fos was expressed at lower levels in all tissues studied. Bone and skin samples had 5-20-fold greater expression than other tissues. Little expression was found in hematopoietic tissues. A single approx 2.2 kilobase transcript of c-fos was identified. Differential expression of c-onc was also found for the c-onc of the Abelson murine leukemia virus v-onc, the Harvey sarcoma virus v-onc, and the Moloney sarcoma virus v-onc. Findings support the idea that c-onc play a role in developmental processes such as cell proliferation and differentiation. (39 Refs)

5.

ski protein product

296. POLYPROTEINS CONTAINING A DOMAIN ENCODED BY THE V-SKI ONCOGENE ARE LOCATED IN THE NUCLEI OF SKV-TRANSFORMED CELLS

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Virology: 151(1):131-8 1986

SKV-transformed nonproducer clones were isolated from infected quail and chicken embryo cells. Analysis of intracellular viral RNAs by the Northern technique revealed that each clone contained a single SKV genome (either 5.7 or 8.9 kb) but no genome of the helper virus. Analysis of intracellular viral proteins containing gag determinants revealed that each clone contained a single species of either 55, 110, or 125 kDa. The intracellular location of these proteins was determined

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by indirect immunofluorescence employing either monoclonal antibodies (anti-p19gag) or conventional antiserum against gag proteins. All three of the SKV-specific proteins were localized to the nuclei of the transformed cells.

297. TRANSFORMING SLOAN-KETTERING VIRUSES GENERATED FROM THE CLONED V-SKI ONCOGENE BY IN VITRO AND IN VIVO RECOMBINATIONS

Stavnezer E, Barkas AE, Brennan LA, Brodeur D, Li Y
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J Virol, 57(3):1073-83, 1986

The Sloan-Kettering viruses (SKVs) are replication-defective retroviruses that transform avian cells in vitro. Each of the three SKV isolates is a mixture of viruses with genomes ranging in size from 4.1 to 8.9 kilobases (kb) with a predominant genome of 5.7 kb. Using a cDNA representing a sequence, v-ski, that is SKV specific and held in common by the multiple SKV genomes, we generated a restriction map of the 5.7-kb SKV genome and molecularly cloned a ski-containing fragment from SKV proviral DNA. Southern hybridization and sequence analysis showed that the cloned DNA fragment consisted of the 1.3-kb ski sequence embedded in the p19gag sequence and followed by the remaining 5' half of the gag gene and small portions of both the pol and env genes. A large deletion encompassing the 3' half of gag and the 5' 80% of pol was mapped to a position about 1 kb downstream from the 3' ski-gag junction. To determine whether the cloned ski sequence had transforming activity, the ski-containing fragment and a cloned Rous-associated virus 1 (RAV-1) genome were used to construct an analog of the 5.7-kb SKV genome, RAV-SKV. Cotransfection of chicken embryo cells with RAV-SKV and RAV-1 yielded foci of transformed cells whose morphology was identical to that induced by the natural SKVs. The transformed transfected cells produced transforming virus with a 5.7-kb ski-containing genome and synthesized a gag-containing polyprotein of 110 kilodaltons (kDa). Several nonproducer clones of RAV SKV-transformed cells were analyzed, and most were found to synthesize a 5.7-kb SKV RNA and a 110-kDa polyprotein. One clone was found to contain an 8.9-kb SKV RNA, and this clone synthesized a 125-kDa polyprotein. Since both the 5.7- and 8.9-kb genomes and the 110- and 125-kDa polyproteins had been identified in studies on the natural SKVs, the present results not only demonstrate the transforming activity of these individual SKVs but also suggest mechanisms for their generation.

298. UNIQUE SEQUENCE, SKI, IN SLOAN-KETTERING AVIAN RETROVIRUSES WITH PROPERTIES OF A NEW CELL-DERIVED ONCOGENE

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J Virol, 57(3):1065-72, 1986

The Sloan-Kettering viruses (SKVs) are a group of transforming retroviruses that were isolated from chicken embryo cells which had been infected with the avian leukosis virus transformation-defective Bratislava 77 (tdB77). Each of the SKV isolates was shown to contain multiple genomes of different sizes indicating the presence of several viruses in addition to tdB77. To identify and characterize the putative transforming gene(s) of the SKVs, we used hybridization selection to isolate the fraction of a representative cDNA which was SKV specific. Both solution and blot hybridization studies with viral RNAs showed that the specific probe contained a sequence, ski, that was at least partially held in common by the multiple SKV genomes. This conclusion was confirmed by the observation that a molecularly cloned ski probe also hybridized to each of the multiple SKV genomes. Southern blots of chicken DNA revealed homologs of ski (c-ski) which were not associated with endogenous viral loci. Results showing that c-ski was expressed in polyadenylated cytoplasmic RNA of uninfected chicken cells indicated that it is a functional gene. Other data showed that c-ski was conserved in avian and mammalian evolution, suggesting a functional role for the gene in species other than chickens. Using ski cDNA in solution hybridizations with viral RNAs and in Southern blot hybridization with cloned retroviral oncogenes, we did not detect any relationship between ski and any of 15 previously identified oncogenes.

299. ONCOGENES OF A NOVEL AVIAN RETROVIRUS AND THREE HUMAN TUMOR CELL LINES

Li Y
Cornell University Medical College
Diss Abstr Int kBI, 44(2):408-B, 1983

Sloan-Kettering viruses (SKVs) are a group of transforming retroviruses which arose spontaneously in ALV tdB77-infected chicken embryo cells. To prepare a probe for the presumptive SKV oncogene, cDNA representative of the sequences in SK770 viral RNAs was hybridized with a large excess of tdB77 RNA. The cDNA which failed to hybridize was shown to be SKV specific by its failure to hybridize with ALV or RSV RNAs and by its efficient hybridization with RNAs from each of the SKV isolates. Hybridization of SKV RNA blots revealed that all of the SKV genomes (but not the tdB77 genome) contain sequences homologous to the SKV-specific probe. Hybridization of the same blot with AV-specific probes showed that each of the SKV genomes also contains ALV-related sequences. Furthermore, restriction enzyme mapping of the SKV 5.7 kb provirus indicated that the ALV sequences in this genome were derived from tdB77. These results support the conclusion that SKVs arose by recombination between tdB77 and a transforming gene, ski, which constitute at least part of the SKV-specific cDNA (cDNAski). Employing cDNAski in solution hybridization with viral RNAs and in Southern blot hybridization with cloned retroviral oncogenes, it is found that ski is not homologous to any of the retroviral oncogenes. Evidence supporting a cellular origin for the SKV ski gene was obtained by Southern blot analysis of DNA from uninfected chicken genome contains homologies of ski (c-ski) which are not adjacent to endogenous viral loci. Further Southern blot analysis of DNAs from several avian and mammalian species using a cloned ski-specific fragment as probe revealed that c-ski is a single gene with large intervening sequences. Its status as a functional gene was supported by the following two experimental results. First, the melting studies of the previous blot revealed a high degree of conservation of c-ski through avian and mammalian species. Second, a ski-specific probe detected two RNA bands on a northern blot of cytoplasmic RNAs from uninfected chicken embryo cells. Southern blots of cloned retroviral oncogenes hybridized with several cloned human tumor cell line probes revealed the following results. (Author abstract)

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II.

STUDIES OF VARIOUS CELLULAR ONCOGENE PROTEIN PRODUCTS

300. SUBSTRATE SPECIFICITIES OF LIPID PHOSPHORYLATION BY TYROSINE KINASES ROS, FPS AND INSULIN RECEPTOR (MEETING ABSTRACT)

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XV Meeting of the European Tumour Virus Group. September 19-24. 1984. Urbino. Italy. p. 117. 1984.

The oncogene product of avian sarcoma virus UR2 (p68v-ros), which belongs to the 'src family' of tyrosine kinases, was recently found to be associated with a phosphatidylinositol (PI) kinase activity upon immunoprecipitation from transformed chick embryo fibroblasts with an anti-gag antibody (Macara et al, Proc Natl Acad Sci, 1984, in press). We have now explored further the phosphorylating activity associated with immunoprecipitates of p68v-ros and p130v-fps (from Fujinami sarcoma virus transformed cells) and with insulin receptor purified from rat hepatocyte membranes, using a variety of substrates. We were unable to detect any insulin-stimulated phosphorylation of PI, phosphatidylinositol phosphate (PIP) or 1,2-diacylglycerol (DAG) with insulin receptor either highly purified by affinity chromatography or as a crude lectin column eluate, under conditions in which insulin-stimulated autophosphorylation was readily apparent. Immunoprecipitates of both p68v-ros and p130v-fps showed PI kinase activity, but we were unable to detect phosphorylation of PIP, DAG or phosphatidyl glycerol. These results demonstrate that the PI kinase activities associated with immunoprecipitated p68v-ros and p130v-fps are highly substrate-specific and are therefore unlikely to be merely adventitious; and also that PI kinase activity is not a property common to all tyrosine kinases. This latter observation might prove useful in distinguishing between different functional classes of oncogene and receptor kinases. (2 Refs)

301. IMMUNOLOGICAL DETECTION OF CELLULAR TARGETS FOR V-ONC GENE CODED TYROSINE KINASES

Giancotti FG. Di Renzo MF. Marchisio PC. Tarone G. Tarone G. Naldini L. Giordano S. Comoglio PM
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Genetic and Phenotypic Markers of Tumors. Aaronson SA. Frati L. Verna R. eds. New York. Plenum Press. p. 297-303. 1984

Immunological detection of cellular targets for v-onc gene-coded tyrosine kinases was studied in cells transformed by Fujinami sarcoma virus (FSV) and feline sarcoma virus of either the Snyder-Theilen (ST-FeSV) or the Gardner-Arnstein strain (GA-FeSV). Onc genes of these viruses (v-fps and v-fes) code for tyrosine phosphokinases that are known to be associated with the plasma membrane and/or the cytoskeleton. Evidence is presented that azobenzyl-phosphonate (ABP) antibodies, used for immunofluorescence microscopy, decorate structures located where plasma membrane and cytoskeleton interact. Immunoprecipitation by ABP antibodies of phosphorylated detergent-insoluble proteins from the following cell sources studied: SR-BALB mouse fibroblasts, SR-A rat fibroblasts, ST-FeSV- and GA-FeSV-transformed Fisher rat embryo cells, and FSV-transformed rat fibroblasts. The ABP antibodies precipitated from each of the transformed lines a phosphotyrosyl protein of mol wt identical to that of the viral oncoprotein: p60 in cells transformed by v-src, pp140 in cells transformed by v-fps, and pp110 in cells transformed by v-fes. These data are consistent with the evidence that kinases coded by onc gene phosphorylate themselves at tyrosine. (13 Refs)

302. CELLULAR ONCOGENES, GROWTH FACTORS, AND CELLULAR GROWTH CONTROL

Pardee AB. Campisi J. Gray HE. Dean M. Sonenshein G
Dana-Farber Cancer Inst., Boston, MA 02115
UT MD Anderson Symp Fundam Cancer Res; 37:21-9 1985

Recent experiments are described that relate expression of proto-oncogenes (normal cellular genes from which viral transforming genes are derived), particularly myc and ras, to the growth regulation of mouse 3T3 fibroblasts. An oncogene may eliminate the requirement for a growth factor, or overexpression of oncogene m-RNA can correlate with tumorigenicity; these and more subtle changes observed in oncogene-containing cells are described. A background review on factors controlling cellular proliferation examines the effect of oncogenes on cellular requirements for external factors such as hormones, growth factors, and nutritional requirements. The intervals of the cell cycle are discussed in light of cell cycle regulation in G1 and cell growth controls with the differences in nontransformed and oncogene-containing transformed cells being emphasized. The role of the myc oncogene in transforming cells and altering cell growth regulation is discussed. The cellular myc proto-oncogene (c-myc) is activated by platelet-derived growth factor (PDGF); other factors must also be present to initiate DNA synthesis. The mRNA for c-myc is produced constitutively in transformed cells but not in nontransformed cells. This suggests that transformation does not require either alterations in the structure of the myc gene or excessive production of its product, but rather a genetic change in the timing mechanism. The role of the ras oncogenes in cell transformation is reviewed and the possible mechanisms underlying this role are discussed. Expression of ras relates to later events in the progression to DNA synthesis; this gene is expressed similarly in both transformed and nontransformed cells. Epidermal (EGF) and insulin-like (IGF I) growth factors are important in initiating DNA synthesis in normal cells but are not required in various transformed cells. The functions of IGF I and EGF in the cell cycle are reviewed. In summary, this chapter relates the functioning of oncogenes, particularly myc and ras, to current ideas regarding regulation of mammalian cell growth by growth factors. Observations regarding the oncogenes, cell cycle-timed events and growth factors are presented along with several theories on the mechanisms by which transformation diminishes growth control, eg, through oncogene interaction with a growth factor or oncogene coding for a growth factor receptor. (39 Refs)

303. BIOCHEMICAL CHARACTERIZATION OF TRANSFORMING-SPECIFIC PROTEINS OF ACUTE AVIAN LEUKEMIA AND SARCOMA VIRUSES

Moelling K. Owada MK. Greiser-Wilke I. Bunte T. Donner P
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J Cell Biochem; 20(1):63-... 1982

The transformation-specific gag-linked fusion proteins of the myelocytomatosis virus (MC29), the avian erythroblastosis virus (AEV), and the Fujinami sarcoma virus (FSV), designated as p110gag-myc, p75gag-erb A, and p130gag-fps, respectively, were analyzed for their cellular localization and biochemical properties after 3,000-fold purification. These polyproteins were analyzed in comparison to the gag-fused polyproteins of replicating viruses that are not directly involved in transformation. Monoclonal antibodies against the viral structural protein p19, which is the N-terminus of the proteins, were used for indirect immunofluorescence, immuno-

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precipitation of the proteins from subcellular fractions, and immunoaffinity column chromatography. Indirect immunofluorescence revealed that the p110 gag-myc was located in the nucleus and bound to DNA after purification. The p75gag-erb A was not nuclear but probably located in the cytoplasm and did not bind to DNA after purification. Neither of the two proteins exhibited protein kinase activity. The p130gag-fps did not bind to DNA but showed protein kinase activity after purification and was not located in the nucleus. Thus, different mechanisms may result in transformation of cells. (17 Refs)

304. REGULATION OF MYC AND RAS PROTO-ONCOGENE EXPRESSION BY EXOGENOUS FACTORS

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Growth and Differentiation of Cells in Defined Environment. Murakami H. Yamane I. Barnes DW. Mather JP. Hayashi I. Sato GH. eds. New York, Springer-Verlag, p. 411-7, 1985.

The relationship between growth-modulating factors and the expression of proto-oncogenes was investigated. Proto-oncogenes are cellular genes which, when mutated or aberrantly expressed, transform certain cells to tumorigenicity. Two proto-oncogenes, c-myc and c-rasKi, show fluctuations in expression that reflect the cellular growth state. The expression of both of these genes diminished when immortalized fibroblasts (murine 3T3) are made quiescent in culture or when embryonal carcinoma cells (murine F9) are induced to terminally differentiate. Findings on the regulations of these two genes during the growth and differentiation of cells in culture are discussed. A growth state dependence for c-myc and c-rasKi expression is demonstrated. The myc gene encodes an approximately 60 kilodalton protein localized in the nucleus, while the rasKi gene encodes a 21 kilodalton protein localized on the inner surface of the plasma membrane. C-myc messenger RNA (mRNA) levels rise early in the growth state transition of the murine F9 cells from quiescence to proliferation and fall early in the terminal differentiation of the cells. By contrast, c-rasKi mRNA abundance rises and falls with later kinetics and to lesser extents. It is known from previous studies that c-myc expression does not directly activate c-rasKi expression, since a transformed 3T3 cell line that expresses c-myc at high levels during quiescence retains the cell cycle dependence of c-rasKi expression. Thus, the expression of these two proto-oncogenes appears to be independently controlled during proliferation. (20 Refs)

305. ONCOGENE EXPRESSION DURING PANCREATIC REGENERATION AND IN CHEMICALLY INDUCED PANCREATIC AND LIVER CARCINOMAS IN THE RAT (MEETING ABSTRACT)

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Fed Proc. 44(5):1493 1985

One objective of this study was to determine which 'oncogenes' are expressed during normal pancreatic growth stimulated by partial pancreatectomy. RNA, isolated from pancreases at various times during regeneration, was examined either by dot blot or 'northern' blot analysis with probes for several oncogenes as well as for 'normal' genes. The expression of the Ha-ras, Ki-ras, myc and raf oncogenes is increased over control levels in regenerating pancreas. This increase precedes the peak of DNA synthesis (72 hr) following partial pancreatectomy and the levels return to normal by 72 hr. Oncogene expression has also been examined in several transplantable rat pancreatic carcinomas and in a primary, rat hepatocellular carcinoma. Dot blot analysis of tumor RNA shows an increase in Ha-ras, Ki-ras, myc and raf expression. Since we observed the most dramatic increase in raf expression, we performed 'northern' blot analysis of these RNA's with a probe for raf. In control liver and in all the tumors examined, there are two transcripts of 3500 and 1800 bases. Control pancreas lacks the 3500 base transcript, but has a 540 base transcript. We have also obtained transformed foci induced by DNA from a pancreatic and a liver tumor in the NIH 3T3 cell transfection/transformation assay. Southern blot analysis of DNA from these foci indicates that raf may be the dominant transforming gene in these tumors.

306. EXPRESSION OF C-RAS AND C-MYC ONCOGENES IN MURINE ERYTHROLEUKEMIAS INDUCED BY FRIEND VIRUSES (MEETING ABSTRACT)

Robert-Lezenes J. Moreau-Gachelin F. Wendling F. Tambourin P. Tavitian A
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XV Meeting of the European Tumour Virus Group. September 19-24, 1984. Urbino, Italy, p. 17, 1984.

We have examined the expression of three cellular oncogenes (c-Ki-ras, c-Ha-ras and c-myc) in different stages of murine erythroleukemias induced in ICFW mice by Friend viruses (F-MuLV and SFFV) in comparison to normal mice spleens and bone marrows, including erythropoietically-stimulated spleens from phenylhydrazine-treated mice. There is no evidence of c-Ki-ras RNA expression in any of the tissues tested. c-Ha-ras RNA was found at similar levels in erythroleukemic cells and normal erythroid cells. In contrast, increased levels of normal 2.3 Kb and short 1.8 Kb c-myc RNA transcripts were detected in both early preleukemic and late leukemic phases of the diseases, as compared to normal erythroid cells. Apparently, neither myc gene amplification nor myc gene rearrangement was observed in erythroleukemias. Our results suggest a possible involvement of c myc either in erythroid cell differentiation or in an early proliferation step of the erythroleukemic process. In this last hypothesis, the Friend viruses might act by an insertional mutagenesis-like mechanism.

307. TRANSLATION OF VIROGENES AND ONCOGENES IN HUMAN LEUKEMIAS AND LYMPHOMAS AND TRANSMISSION OF CODING DNA (MEETING ABSTRACT)

Toth FD. Vaczi L. Szabo B. Kiss J. Kiss A. Rak K. Kovacs I. Kiss C
Inst. Microbiol., Med. Univ. Sch., 4012 Debrecen, Hungary

XV Meeting of the European Tumour Virus Group. September 19-24, 1984. Urbino, Italy, p. 7, 1984.

We have examined the translational expression of antigens related to p30 polypeptides of baboon endogenous virus (BaEV) and gibbon ape leukemia virus (GaLV) and proteins encoded by myc and src oncogenes by immunofluorescence in human leukemia and lymphoma cells. Most samples irrespective of cell type expressed BaEV p30-like antigen. In contrast expression of antigen related to GaLV p30 was detected in acute myeloid leukemia, blastoid phase of chronic granulocytic leukemia and in B- and O-cell lymphoid malignancies. Translational expression of myc gene showed lack of specificity. Expression of src was relatively low in most samples, with markedly elevated levels in a few leukemias and lymphoma samples. Transfection of NIH 3T3 cells was performed by the calcium phosphate precipitation technique. Transformed foci expressed both myc and src-specific proteins and very frequently a GaLV p30-like antigen, too.

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308. STIMULATION AND INHIBITION OF GROWTH BY EGF IN DIFFERENT A431 CELL CLONES IS ACCOMPANIED BY THE RAPID INDUCTION OF C-FOS AND C-MYC PROTO-ONCOGENES

Brata R. Burckhardt J. Curran T. Muller R
European Molecular Biology Laboratory. Heidelberg. FRG.
EMBO J. 4(5):1193-7 1985

Stimulation of quiescent fibroblasts to growth by polypeptide growth factors is accompanied by the rapid induction of c-fos and c-myc proto-oncogenes. In contrast to fibroblasts, A431 cells respond to epidermal growth factor (EGF) with a decreased growth rate. Here we report that, in spite of its growth inhibitory effect, EGF rapidly induces transient expression of c-fos mRNA, followed by the synthesis of nuclear c-fos protein. In addition, EGF treatment resulted in elevated levels of c-myc expression. Practically identical results were obtained with variant A431 clones that are resistant to the inhibitory effect of EGF on cell proliferation. These observations suggest that in A431 cells c-fos and c-myc induction is a primary consequence of growth factor-receptor interaction. Indeed, efficient induction of both genes was also observed with cyanide bromide-cleaved EGF, which has previously been shown to be non-mitogenic but able to trigger early events induced by EGF. We observed strong induction of c-fos and to a lesser extent of c-myc also by TPA, and by the calcium ionophore A23187, indicating an important role for kinase C in proto-oncogene activation by growth factors.

309. TISSUE-SPECIFIC EXPRESSION OF CELLULAR ONCOGENES IN DEVELOPING HUMAN FETUS OF THE FIRST TRIMESTER (MEETING ABSTRACT)

Pfeifer-Ohlsson S. Rydnert J. Goustin A. Larsson E. Ohlsson R
Dept. of Oncology. S-901 87 Umea. Sweden
XI' Meeting of the European Tumour Virus Group. September 19-24. 1984. Urbino. Italy. p. 11. 1984.

Primarily due to work on transforming retroviruses a set of cellular genes with transforming properties when residing in a retroviral genome has been isolated. These oncogenes have also been implied to play a role in human tumorigenesis in fashions reminiscent of the viral oncogenes. As the oncogenes appear to be evolution-conserved it has been argued that they play important roles in normal cell metabolism. Moreover, the transforming properties suggest a role for such oncogenes in the control of differentiation or proliferation of normal cells. We have therefore undertaken a survey of c-oncogene expression in normal embryonal cells for the following reasons: (1) a distinct congruence between the spatial and temporal pattern of a developmental event and the distribution of active c-oncogenes may provide insight into both the normal snf transforming functions of particular oncogene. (2) Once such functions have been defined, the active c-oncogenes may turn out to serve as endogenous markers for specialized functions and possibly as markers for different cell-cycle stages. Using in situ hybridization techniques to thin sections of formalin-fixed human fetus we have analyzed the distribution of active c-fos and c-myc oncogenes. The results can be summarized as follows: The c-fos transcripts are found mainly in the developing bone tissue but also, to a lesser degree, in epidermis. The c-myc transcripts are found mainly in the primitive epidermis but also in connective tissue and gut epithelial layers. The data reveal further that c-oncogene expression appears to be synchronized in only one plane. The results are consistent with a role for c-oncogenes in normal developmental p rocesses.

310. TRANSLATION OF ONCOGENES IN MYELO- AND LYMPHOPROLIFERATIVE DISEASES (MEETING ABSTRACT)

Toth FD. Vaczi L. Kiss J. Szabo B. Kiss A. Rak K
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XXIIth Symposium for Comparative Research on Leukemia and Related Diseases. July 7-11. Hamburg. p. 81. 1985.

Fresh human leukemia and lymphoma cells were examined for expression of myb, myc, src and ras oncogenes. Expression of the myc gene showed lack of specificity. Expression of src was elevated in a few cases of T-cell tumors and myeloid leukemias. Nuclear protein encoded by the myb gene was found in all samples from acute and chronic myeloid leukemias. Expression of the ras-specific proteins was observed in acute myeloid leukemia, in the blastoid crisis of chronic myeloid leukemia and in some B-cell malignancies. Transforming DNA was transfected into NIH 3T3 cells. Transfection was performed by the calcium phosphate precipitation technique. Transformed foci expressed proteins, encoded by the onc genes, active in the human leukemia or lymphoma samples. Data suggest cooperation between myb and ras oncogenes in acute myeloid leukemia, and between myc and ras oncogenes in some B-cell malignancies. Similar interaction between myb and src, as well as between myc and src oncogenes, seem probable in a few cases of chronic myeloid leukemia and T-cell malignancies, respectively.

311. COEXPRESSION OF THE SIS AND MYC PROTO-ONCOGENES IN DEVELOPING HUMAN PLACENTA SUGGESTS AUTOCRINE CONTROL OF TROPHOBLAST GROWTH

Goustin AS. Betsholtz C. Pfeifer-Ohlsson S. Persson H. Rydnert J. Bywater M. Holmgren G. Heldin CH. Westermark B. Ohlsson R
Unit for Applied Cell and Molecular Biology. University of Ume+a. Sweden.
Cell. 41(1):301-12 1985

First trimester human placentas actively express the sis proto-oncogene, the structural gene for the B chain of platelet-derived growth factor (PDGF). Using the in situ hybridization technique, the 4.2 kb c-sis transcript has been localized to the cytotrophoblastic component, especially the highly proliferative and invasive cytotrophoblastic shell, paralleling the distribution of c-myc transcripts in early placenta. Explants of first trimester placenta release significant levels of PDGF-like activity into the medium under apparent developmental control. Moreover, cultured trophoblasts display abundant high-affinity PDGF receptors and respond to exogenous authentic PDGF by an activation of the c-myc gene and DNA synthesis. The developing human placenta may therefore represent a case of autocrine growth regulation in a normal tissue, in which cells bearing receptors for a growth factor can also synthesize and respond to that factor.

312. ONCOGENE ENCODED PROTEINS: HUMAN IMMUNOANATOMIC DISTRIBUTION AND TUMOR IMMUNOPATHOLOGIC EXPRESSION (MEETING ABSTRACT)

Cordon-Cardo C. Niman HL. Furth ME. Morrissey DM. Melamed MR. Old LJ. Lerner RA
Memorial Sloan Kettering Cancer Center. NY. NY 10021
Proc Annu Meet Am Assoc Cancer Res. 26:64 1985

A panel of antibodies (Abs) of predetermined specificity, detecting proteins encoded by human oncogenes, has been used to analyze the expression of the antigens recognized in cultured cell lines and in normal and neoplastic human tissues. This panel included Abs (monoclonal and polyclonal) to Ras, Sis, Myc, Myb, Abl and Fes. Antigen distribution has been defined by reactions with human cultured cell lines,

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frozen and paraffin sections of normal adult and fetal tissues and with human tumor tissues. Specificity was determined by blocking tests with the relevant peptides. The Abs were all found to be broadly reactive with cultured cell lines. Their patterns of reactivity in normal tissues ranged from intermediate (Ras, Sis, Abl) to very restricted (Myc, Myb, Fes). Ras was expressed by placental trophoblast, epithelial cells of proliferative compartments (ie: basal cells of the epidermis), and subset of neurons; Sis was detected in megakaryocytes, platelets, and colonic epithelium; Abl was found in some proliferative compartments; Abl and Myc subset lympho-hematopoietic cells and neurons; Fes was detected in rare mesenchymal cells. Myb was not found in any normal tissues. The Abs presented distinct patterns of reactivities with tumors, suggesting possible application as markers for immunodiagnosis and/or clinical imaging.

313. EFFICIENT GENERATION OF ANTIBODIES TO ONCOPROTEINS BY USING SYNTHETIC PEPTIDE ANTIGENS

Tanaka T. Slamon DJ. Cline MJ
Department of Medicine. University of California School of Medicine. Los Angeles 90024.
Proc Natl Acad Sci USA: 82(10):3400-4 1985

To examine the efficiency of generating protein-reactive antipeptide antibodies, 35 peptides encoded by retroviral or cellular oncogenes were used to immunize rabbits. Thirty-two peptides elicited antipeptide antibodies, of which 56% reacted with their respective oncoproteins. The length of the immunizing peptide was an important factor in generating antibodies reactive with native protein. Similar peptides differing in a single or a few amino acids could elicite antisera of markedly different reactivities.

314. DETECTION OF ONCOGENE-RELATED PROTEINS IN THE URINE OF CANCER PATIENTS BY SEQUENCE-SPECIFIC ANTISERA (MEETING ABSTRACT)

Niman HL
Scripps Clinic and Research Foundation. CA 92037
Hybridoma. 4(1):75 1985

Synthetic peptides representing the predicted amino acid sequence of oncogenes have been used to generate polyclonal and monoclonal antibodies. Antisera which recognize both the synthetic peptide and the oncogene protein product have been used to screen the urine of normal, pregnant, and cancer patients. The urine has been screened using Western blotting techniques to determine the amount and mole cular weight of oncogene-related proteins. The urine was concentrated, dialyzed and electrophoresed into a polyacrylamide gel and onto nitrocellulose. The concentrate was then probed with appropriate sequence-specific antisera. The specificity of the reaction was then confirmed by blocking the binding activity with the synthetic peptide. This approach has detected proteins related to ras, sis, and fes in normal and cancer patients. Protein size and concentration differences have been noted among the proteins detected in the two populations. These differences and results using additional samples and antisera were discussed.

III.

REVIEWS OF OTHER RELATED STUDIES

315. CELLULAR ONCOGENES AND THE PATHOGENESIS OF CANCER

Weinberg RA
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Concepts in Viral Pathogenesis. Notkins AL. Oldstone MBA. eds. New York. Springer-Verlag. pp. 178-86 1984

Certain normal cellular genes, called 'proto-oncogenes', exhibit an oncogenic potential after activation by one of two strategies, namely, retrovirus retrieval or gene transfer. The retrovirus retrieval is exemplified by the results of molecular analysis of the genome of the retrovirus Rous sarcoma virus, whereby one part of the genome was found to consist of viral genes that are necessary for growth of the virus and a second part to consist of the oncogene used by the virus to transform infected cells; the oncogene was found to have its origin in the genome of the normal cell. The gene transfer is exemplified by the finding that when DNAs from tumors of largely nonviral origin were applied to monolayer cultures of NIH3T3 mouse fibroblasts by gene transfer procedures, foci of transformed cells appeared, signalling the presence of transforming sequences in the applied donor DNA. Mechanisms whereby proto-oncogenes become converted into active oncogenes are presented and discussed under the following section headings: Activation by alteration of encoded protein, enhancement of expression by promoter replacement, co-transcription of cellular genes with inserted promoters, and activation via enhancement. In many animals, retroviruses may activate cellular proto-oncogenes by transduction or by adjacent integration; however, in humans bearing tumors of suspected or known viral etiology, the role of viruses in creating oncogenes is still quite obscure. (29 Refs)

316. CELLULAR ONCOGENES AND MULTISTEP CARCINOGENESIS

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Science: 222(4625):771-8 1983

Two dozen cellular proto-oncogenes have been discovered to date through the study of retroviruses and the use of gene transfer. They form a structurally and functionally heterogeneous group. At least five distinct mechanisms are responsible for their conversion to active oncogenes. Recent work provi des experimental strategies by which many of these oncogenes, as well as oncogenes of DNA tumor viruses, may be placed into functional categories. These procedures may lead to definition of a small number of common pathways through which the various oncogenes act to transform cells. (74 Refs)

317. MUTATIONS OF CELLULAR ONCOGENES AS A BASIS FOR NEOPLASTIC CHANGE

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Environ Sci Res: 31:61-74 1984

The identification and activation of cellular oncogenes, the relationship of insertional mutagenesis to oncogenesis, the role of proviruses in oncogenesis, and other genetic rearrangements which affect cellular oncogenes are reviewed. Many tumorigenic retroviruses do not carry oncogenes; instead, retroviral proviruses act as insertional mutagens to augment ex-

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pression of cellular genes. Some activated genes are in the c-onc family, but others, eg, mammary carcinoma induced by the mouse mammary tumor virus, involve proviral insertion mutations that affect cellular genes not represented among retroviral oncogenes. Retroviral DNA is able to activate expression of cellular genes by at least two mechanisms: the provision of a strong viral promoter element for transcription of 'downstream' genes or the indirect action of a viral 'enhancing' element upon cellular sequences competent to serve as transcriptional initiation sites. Translocations occur commonly in tumor cells. Sometimes the break points for the translocations are located nearby and even with c-onc loci, including c-myc. Gene amplification is frequently found in tumor cells. In several human and murine tumor lines the amplified domains have been shown to include c-onc genes that are also expressed at a correspondingly high level, again, with c-myc involvement. DNA from human tumors and tumor cell lines can often transform cultured mouse cells; the transforming activities have been identified in several cases as mutated versions of c-onc's. If a number of mutant genes contribute in concert to tumorigenesis, it may be extremely difficult to determine the function of each mutation in the neoplastic process. (45 Refs)

318. EVOLUTION OF CELLULAR ONCOGENES.

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Dept. of Virology, Weizmann Inst. of Science, Rehovot, Israel
Adv Viral Oncol. 4:29-44 1984

The vertebrate genome contains a set of cellular genes which can be converted into transforming genes (oncogenes). Current ideas and conclusions on the evolution of oncogenes are highlighted and the distribution of cellular oncogenes (c-onc) in different species and phyla is emphasized. The implications on the function of the gene products, on the essential domains of the c-onc proteins, and on the interaction of the oncogenes with other cellular proteins are discussed. The c-oncs discussed are src, mos, fes-fps, abl, has and myc. Also considered are the structural and functional conservation of cellular oncogenes. The identification of Drosophila genes which are structurally similar to vertebrate oncogenes provides a mechanism to identify the function of these genes. Through sophisticated genetic manipulations available in Drosophila it should be possible to determine whether cellular oncogene products are essential during development and in which tissues they are required. (79 Refs)

319. AMPLIFICATION OF CELLULAR ONCOGENES IN CANCER CELLS

Alitalo K
Department of Virology, University of Helsinki, Finland
Med Biol. 62(6):304-17 1984

Regulatory or structural alterations of cellular oncogenes have been implicated in the causation of various cancers. Oncogene alteration by point mutations can result in a protein product with strongly enhanced oncogenic potential. Aberrant expression of cellular oncogenes may be due to tumor-specific chromosomal translocations that dysregulate the normal functions of a proto-oncogene. Amplification of cellular oncogenes can also augment their expression by increasing the amount of DNA template available for the production of mRNA. It appears that amplification of certain oncogenes is a common correlate of the progression of some tumours and also occurs as a rare sporadic event affecting various oncogenes in different types of cancer. Amplified copies of oncogenes may or may not be associated with chromosomal abnormalities signifying DNA amplification: double minute chromosomes and homogeneously staining chromosomal regions. Amplified oncogenes, whether sporadic or tumour type-specific, are expressed at elevated levels, in some cases in cells where their diploid forms are normally silent. Increased dosage of an amplified oncogene may contribute to the multistep progression of at least some cancers. (91 Refs)

320. RETROVIRAL AND HUMAN CELLULAR ONCOGENES

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Ann Clin Lab Sci. 14(5):343-54 1984

Unexpected meeting of two separate lines of research resulted in the discovery of oncogenes. Oncogenes are deoxyribonucleic acid (DNA) sequences coding for polypeptide gene products which cause, or contribute to, neoplastic growth of cells. Oncogenes remain almost unchanged through evolution: oncogenes and their gene products of avian, murine, feline, simian and human species show close homology. Retroviruses possess three genes encoding virion structural proteins and envelope. The DNA copy of the viral genome (the provirus) recombines with DNA sequences of the host cell genome and thus acquires an additional DNA sequence of host origin (transduction). The newly acquired DNA sequences render the retrovirus oncogenic. Certain genomic DNA sequences extracted from human tumor cells induce malignant transformation in selected assay systems (transfection). The transforming genes of retroviruses show close homology to these cellular oncogenes. Retroviruses appear to have acquired cellular proto-oncogenes during past interactions with their host cells. In the cell, proto-oncogenes are presumed to fulfill fundamental functions of cell differentiation and mitosis. This is deduced from their preservation during evolution, i.e., proto-oncogenes of avian, lower and higher mammalian and human species display close DNA sequence homology and thus their gene products are also similar in distant species. When expressed in excess or in altered form or at a wrong chromosomal location or at an inappropriate time of the cell cycle, proto-oncogenes function as oncogenes by inducing mitoses and inhibiting differentiation of their host cells. (64 Refs)

321. ONCOGENES AND HUMAN NEOPLASIA

Pitot HC
Department of Oncology, McArdle Laboratory for Cancer Research, Medical School, University of Wisconsin, Madison.
Clin Lab Med. 6(1):167-79 1986

Genes whose products are directly involved in the transformation of a normal to a neoplastic cell are present in most but not all oncogenic RNA viruses (retroviruses) have genes found in oncogenic RNA viruses (retroviruses) have also been found in normal cells, their coding sequences highly conserved in evolution. Such viral genes (v-onc) are expressed at high levels in infected cells. 'Activation' of cellular homologues of v-onc genes (c-onc) may occur by a variety of mechanisms leading to an abnormal and/or increased expression of such activated c-onc genes in malignant cells. Although oncogene activation appears to be a critical step in the neoplastic transformation induced by oncogenic viruses, the role of this process in the development of chemical and radiation-induced neoplasia is not yet clear. (56 Refs)

322. ONCOGENES

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Mayo Clin Proc. 60(10):697-713 1985

Oncogenes were first identified in neoplastic tissues and were thought to be disseminated by retroviruses. They were

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shown to cause neoplastic transformation of cells in vitro and were therefore regarded as 'the genes that cause cancer.' They occur in all eukaryotes including humans. More than 30 oncogenes have been identified, and 28 have already been mapped to the human karyotype. When the protein products of the oncogenes were investigated, they were found to be very similar to known substances that are normally involved in the control of cell division - growth factors, plasma-membrane receptors, modulators of the transduction of exogenous signals through the plasma membrane, and nuclear DNA-binding substances. These discoveries changed the original concept of oncogenes. They are now regarded as playing vital roles in the normal control of mitosis, and they should properly be called mitogenes rather than 'oncogenes.' Like all other genes, if a mitogene is mutated, rearranged, translocated, or otherwise deranged, its effects will change; it may then become an oncogene that will pro mote disorderly cell division and thus contribute to the pathogenesis of cancer. In addition to providing new insights into the normal control of mitosis and its neoplastic transformation, the discovery of the mitogenes has suggested new approaches to the early diagnosis of cancer, to a more rational classification of neoplasms based on pathogenesis rather than morphology, and, perhaps, to more rational forms of therapy. (39 Refs)

323. ONCOGENES IN RETROVIRUSES, MALIGNANCY, AND NORMAL TISSUES

Lacey SW
University of Alabama, Birmingham.
Am J Med Sci; 291(1):39-46 1986

Oncogenes are genes with a proven cancer association that appear to function primarily in the regulation of cellular proliferation and differentiation. The preliminary work leading to their discovery, their association with retroviruses, the retrovirus life cycle, and the use of retroviruses to find and characterize oncogenes is discussed in detail. Chromosomal abnormalities are discussed using Burkitt's lymphoma and the myc oncogene as prototypes. Finally, the normal (nonmalignant) functioning of oncogenes is discussed in terms of embryogenesis and hepatic regeneration. (23 Refs)

324. RETROVIRAL ONCOGENES AND THEIR CELLULAR PROTO-ONCOGENES

Zaharetskii ER
Mol Biol (Mosk); 19(1):9-35 1985

Recent data on the genome structure of transforming retroviruses and their cellular counterparts has been reviewed. Retroviruses are divided in several groups according to their oncogenic potential. Comparison of oncogenes and their protein products for most abundant transforming viruses are presented. Probable mechanisms of capture of cellular proto-oncogenes by retroviruses and the hypothesis of existence of endogenous transforming viruses are discussed. General features of cellular proto-oncogenes and possible ways of their activation resulting in neoplastic transformation are discussed. Some unresolved problems of retroviral carcinogenesis, in particular, the problem of existence of unidentified 'X'-genes in retroviral genomes and involvement of constitutional viral genes in carcinogenesis are mentioned. (139 Refs)

325. ONCOGENES: IMPLICATIONS FOR THE DIAGNOSIS AND TREATMENT OF CANCER

Cline MJ, Slamon DJ, Lipsick JS
Department of Medicine, UCLA School of Medicine.
Ann Intern Med; 101(2):223-33 1984

Cellular oncogenes comprise a small family of genes, highly conserved throughout vertebrate evolution, that code for proteins with diverse functions including DNA binding, protein kinase, and cellular growth factor activities. Cellular oncogenes are important in certain aspects of the proliferation and differentiation of normal cells. Under some circumstances these genes may also induce malignant transformation of normal cells. Various mechanisms may underlie their involvement in carcinogenesis. Incorporation of all, or part of, cellular oncogenes into RNA tumor viruses, mutations in gene stru cture, or translocation of cellular oncogenes from one chromosome to another may all be associated with the induction of malignant change in cells. In some of these situations altered oncogene products are made. Knowledge about the biology of oncogenes may lead to improved techniques for cancer detection and perhaps new approaches to cancer treatment. (88 Refs)

326. ROLE OF ONCOGENES IN THE GENESIS OF HEMOBLASTOSES

Iavorkovskii LI
Eksp Onkol; 7(3):12-20 1985

The recent data on cellular oncogenes and their significance in the origin of leukemias and lymphomas are reviewed. A brief historical note of the discovery of cellular oncogenes on the example of the Rous sarcoma virus and its cellular homologue is presented. The connection between chromosome translocations and oncogene activation as well as implication of carcinogens and genetic factors is elucidated. The proteins encoded by cellular oncogenes are described and possible pathways of their transforming activity are analyzed. (37 Refs)

327. HUMAN ONCOGENE-RELATED PROTEINS IN URINE DURING PREGNANCY AND NEOPLASIA

Niman HL
Department of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla, California.
Clin Lab Med; 6(1):181-96 1986

The use of site-directed monoclonal antibodies for the detection of oncogene-related proteins in human urine samples is described. This approach identifies differential elevations of such proteins in the urine of various cancer and pregnant patients. (103 Refs)

328. REPORT ON THE CONFERENCE 'ONCOGENES CELL GROWTH AND CANCER'

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Report on the Conference 'Oncogenes Cell Growth and Cancer', Gitol D, 1984.

A report on the conference 'Oncogenes Cell Growth and Cancer' held at the NIH (November 14-16, 1984) is presented. Sessions were held on the following topics: the ras oncogenes and malignancy; growth factors, receptors and oncogenes; oncogene activation by chromosomal translocation; steps in cell transformation; and protein kinase in normal and malignant growth. New oncogenes, classification of the oncogenes, cellular functions of oncogenes, mechanisms of malignancy, and prospects for applications are discussed in the report. Several new cellular oncogenes were reported, including oncogene L-myc, identified in small cell lung carcinoma; oncogene met, described in the chemically transformed osteogenic sarcoma line; oncogene neu, isolated from NIH 3T3 foci formed by transfection with DNA from ethylnitrosourea-induced rat neuroblastoma; the gene coding for the well studied tumor-associated antigen p53; and two possible oncogenes, bcl-1 and bcl-2, detected in various B cell leukemias. Four classes of oncogenes were defined: kinase associated, the ras family, nuclear oncogenes and growth factor related. The most telling new information on cellular functions of oncogenes concerns the pos sible func-

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tion of c-ras; one ras gene was shown to be essential for the germination of yeast. Serial concepts on the mechanism of malignancy were presented.

329. MALIGNANT CELL TRANSFORMATION AND ONCOGENES

Kiselev FL
Genetika; 21(6):885-95 1985

In genome of all transforming retroviruses special genes (oncogenes) have been identified which play a key role in malignant conversion of the cells, infected with these viruses. The homologues of these genes (protooncogenes) are persist in all normal cells. During transformation protooncogenes can be activated as a result of one of following processes: insertion of promotor-like elements, mutations, translocations, amplifications or rearrangements. Using transfection technique the transforming genes were isolated from different human tumors. The activation of one of the cellular oncogenes may switch on the other genes and malignant cell transformation may be characterized as a multifactor and multistage process. (46 Refs)

330. ONCOGENE EXPRESSION AND CELL TRANSFORMATION

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Gan To Kagaku Ryoho; 10(2 Pt 2):512-9 1983

Recent studies on transforming genes of retroviruses have revealed that these viral transforming genes (v-onc) were derived from prototype genes (c-onc) present in normal cells. These c-onc genes are believed to carry out some unknown but important functions for cells to proliferate or differentiate. As a plausible mechanism of carcinogenesis it has been proposed that cells in which one of these onc genes happen to be expressed to abnormal extent as a result of mutations in controlling elements or as a result of infection with viruses containing these genes would become cancerous. In this article, the common features of products of genetically different viral onc genes found in avian sarcoma viruses and their cellular onc genes are described. Experiments of recovered ASV suggesting close relationship between viral and cellular genes and studies on mechanism of cell transformation by these viral gene products are also presented. (20 Refs)

331. ONCOGENES, IONS, AND PHOSPHOLIPIDS

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Department of Radiation Biology and Biophysics, University of Rochester, School of Medicine and Dentistry, NY.
Am J Physiol; 248(1 Pt 1):C3-11 1985

Recent discoveries in tumor virology, lipid biochemistry, and ion transport studies promise to revolutionize our understanding of cell proliferation, differentiation, and tumorigenesis. A model is proposed, based on similar schemes presented recently by others, that incorporates these discoveries and provides a focus for future research on the functions of oncogene proteins. The model suggests that the early (competence) events in the initiation of cell proliferation are triggered by activation of phosphatidylinositol (PI) turnover, which releases two second messengers, 1,2-diacylglycerol (1,2-DG) and inositol-1,4,5-trisphosphate (IP3). PI turnover is proposed to be regulated by the oncogene protein kinases (src, ros, abl, fps) either directly (acting as PI kinases) or indirectly (as tyrosine kinases). The IP3 triggers Ca2+ release from internal stores, and the elevation of cytosolic Ca2+ acts synergistically with 1,2-DG to activate the Ca2+- and phospholipid-dependent kinase C. Kinase C copurifies with the receptor for the tumor-promoting phorbol esters. It is suggested that kinase C then activates the Na+-H+ exchange system, resulting in an elevation of cytosolic pH and Na+, and that these ionic signals (including the change in Ca2+), either in concert or individually, induce further events, including expression of the protooncogene c-myc, which together commit the cell to initiate replication. Evidences in support of this model are reviewed, together with complications indicating its present inadequacies, particularly recent data suggesting that 1,2-DG may activate tyrosine kinases independent of kinase C. (93 Refs)

332. CANCERS, ONCOGENESIS AND ONCOGENES

Meyer G, Nicoli J
U 119 INSERM - Institut J.-Paoli et J.-Calmettes, Marseille.
Med Trop (Mars); 43(3):217-25 1983

The carcinogenic transformation depends on the presence of particular genes, the oncogenes, which are, as a matter of fact, normal cellular genes. The oncogenic retro-viruses are mosaic genomes, transducing oncogenes in the infected cells. These genes are expressed: the product has an activity of protein kinase. The expression is increased in the transformed cells, because the oncogenes are either linked to strong viral promoters in the oncogenic viruses, or translocated in the vicinity of immunoglobulin genes in the case of Burkitt lymphoma. Moreover, there is some modifications (punctual mutations or deletions) of the primary sequence of the protein. (32 Refs)

333. ONCOPROTEINS - THE PRODUCTS OF ONCOGENES. TYROSINE PROTEIN KINASES

Kiselev LL
Mol Biol (Mosk); 19(2):309-32 1985

The review summarizes the data mostly published in 1982 and 1983 concerning isolation, identification, cellular localization, deciphering of primary structure, and properties of the proteins coded by the retroviral oncogenes. A tentative classification of oncoproteins is suggested. The main part of the review is devoted to oncoproteins with tyrosine specific protein kinase activity. A hypothesis is proposed concerning the possible mode of action of these proteins on transforming cells. It is stressed in conclusion that the remarkable progress of our knowledge in this field has been mostly achieved due to recombinant DNA technique successfully and widely applied for studying oncogene structure and function. (195 Refs)

334. PROTEIN PHOSPHORYLATION AND GROWTH CONTROL

Hunter T, Alexander CB, Cooper JA
Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California 92138.
Ciba Found Symp; 116:188-204 1985

Many growth factor receptors and retroviral transforming proteins share the property of phosphorylating proteins on tyrosine. Several substrates for both types of protein-tyrosine kinase have been identified. Treatment of quiescent cells with growth factors such as EGF and PDGF, whose receptors have ligand-stimulated protein-tyrosine kinase activities, induces tyrosine phosphorylation of three proteins, p45, p42 and p41. Two phosphorylated forms of p42 are found, the more basic of which is present in some but not all cells transformed by viral protein-tyrosine kinases. p42 is rapidly (as early as 1 min) but transiently (decreased to baseline by 2h) phosphorylated following PGDF or EGF treatment of quiescent fibroblasts. At saturating levels of mitogen the stoichiometry of p42 phosphorylation is greater than 50%. p42 is a highly conserved, rare (0.002% of total cell protein), soluble cytoplasmic protein. IGF I and insulin, whose receptors also have ligand-stimulated protein-tyrosine kinase activity, induce p42 phosphorylation in appropriate cells. In the case of insu-

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lin this effect has been observed in cells with large numbers of insulin receptors. p42 is also phosphorylated in response to mitogens whose receptors lack protein-tyrosine kinase activity, for example 12-O-tetradecanoylphorbol-13-acetate (TPA) and thrombin. For TPA there is evidence that this is an indirect effect due to the activation of a protein-serine/threonine kinase. On the basis of the highly conserved nature of this response and its generality, it seems likely that tyrosine phosphorylation of p42 is important for at least early responses to mitogens.

335. VIRUS- AND CELL-ENCODED TYROSINE PROTEIN KINASES INACTIVATE DNA TOPOISOMERASES IN VITRO

Tse-Dinh YC, Wang TW, Goldberg AR
Central Research and Development Department, E. I. du Pont de Nemours and Co., Wilmington, Delaware
Nature, 312(5990):785-6 1984

Tyrosine protein kinase activity is associated with at least eight different retrovirus-encoded onc gene products and with cell receptors for epidermal growth factor, platelet-derived growth factor, tumour growth factor and insulin. Both the onc kinases and the growth factor receptors are membrane proteins whose enzymatic activity has been implicated in stimulation of growth. However, the mechanism by which a signal passes from the plasma membrane to the nucleus to initiate growth remains unknown. As DNA topoisomerases catalyse the interconversion of topological isomers of DNA and hence affect DNA replication, transcription and recombination, they may be involved also in stimulation of growth. Several DNA topoisomerases have been shown to form a covalent complex with DNA via a phosphotyrosine linkage. The DNA-protein complex is postulated to be an intermediate in breaking and rejoining of DNA. The aim of the present study was to determine whether tyrosine protein kinases modulate the activity of topoisomerases by phosphorylating the tyrosine residue involved in DNA binding. We report that incubation of Escherichia coli and calf thymus type I DNA topoisomerases with the Rous sarcoma virus transforming gene product, pp60src, and TPK75, a tyrosine protein kinase purified from normal rat liver, results in a 10-fold loss of topoisomerase activity.

336. ROLE OF THE ONCOGENES AND THE PROTEIN KINASES IN CARCINOGENESIS

Kahn A, Tuy FP
Institut de Pathologie Moleculaire, INSERM U. 129, C.H.U. Cochin, Paris
Presse Med, 12(34):2101-5 1983

The genome of higher animals contains genes called cOnc which are thought to be responsible for cancer when activated. Many oncogenic retroviruses also contain vOnc genes responsible for their transforming properties. cOnc sequences are homologous and result, in fact, from genetic recombinations between retroviruses lacking transforming genes and the animal genome. Onc genes encode transforming proteins which are biological intermediates of their carcinogenic property and may consist of protein kinases active on membrane and cytoskeleton constituents or nuclear proteins probably active on DNA. Modifications of cOnc genes (or oncogenes) due, in particular, to chromosomal rearrangements and occasional mutations have been demonstrated in an increasing number of human cancers.

337. TYROSINE PHOSPHORYLATION AND ONCOGENES

Newmark P
Nature, 4 Little Essex St., London WC2R 3LF, England
Nature (Lond), 292(5818):15-16 1981

Topics discussed at the Cold Spring Harbor RNA Tumor Virus Meeting are summarized, with emphasis on the report of the existence of a cascade of cellular kinases, the activation of any member of which depends on phosphorylation of a tyrosine residue contained therein. Members of the cascade are themselves substrates for tyrosine phosphorylation by products of transforming genes of RNA tumor viruses. The findings, if substantiated, bring a new perspective to the actions of RNA tumor viruses and may indicate the means by which products of transforming genes convert a normal cell into a tumor cell. (no Refs)

338. ROLE OF GROWTH FACTORS IN ONCOGENESIS: GROWTH FACTOR-PROTO-ONCOGENE PATHWAYS OF MITOGENESIS

Huang JS, Huang SS
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Ciba Found Symp, 116:46-65 1985

Cellular genes which encode proteins involved in the response of cells to stimulation by growth factors may be potential oncogenes. The factors involved in the signal transmission from growth factor-receptor interaction to DNA synthesis constitute a cascade system which we call the 'growth factor-proto-oncogene pathway(s) of mitogenesis'. For each growth factor, all the responsive cells, regardless of cell types and tissue source, have specific growth factor receptors which are similar, if not identical, in molecular weight and biological activity. Thus, we believe that the growth factor-proto-oncogene pathway(s) functions in the same manner in all responsive cells. Platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and brain-derived growth factor (BDGF) are major growth factors for connective tissue cells and do not share a common pathway in mitogenesis in responsive cells. The gene product of c-myc may be involved in the cellular response of cells stimulated by PDGF or FGF, but not directly in the signal transmission which leads to DNA synthesis.

339. HUMAN PROTO-ONCOGENES, GROWTH FACTORS, AND CANCER

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NCI, Bethesda, MD 20205
UT MD Anderson Symp Fundam Cancer Res, 37:241-55 1985

In this review, studies are summarized that have led to identification of the first normal cellular function of a proto-oncogene, and evidence is presented which suggests how this gene may be involved in certain kinds of human cancer. Also described are investigations of another family of proto-oncogenes that have been more firmly implicated as human transforming genes, but whose normal function remains to be determined. The sis-oncogene was first identified as the transforming gene of the simian sarcoma virus (SSV). The cloning of biologically active SSV DNA and the detailed analysis of this viral genome, with attention to the v-sis-oncogene segment and its origin in terms of species, is reviewed here. Discussion of the SSV includes the requirement of v-sis for SSV oncogenic activity, nucleotide sequence analysis of SSV, identification of the SSV transforming protein (the sis-encoded protein) and its function, and the relationship of amino acid sequences in SSV-transforming protein to those in human platelet-derived growth factor (PDGF). Addressing this last point, work is described which attempts to establish directly that the v-sis gene product and human PDGF

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share structural, immunologic. and biologic properties. Results of attempts to define, at the molecular level, the role of the sis proto-oncogene in human malignancies are presented along with attempts to characterize the sis/PDGF-2 locus. Nucleotide sequence analysis suggested that c-sis (human) is the structural gene for PDGF-2. Data are presented which establish that the normal human gene encoding PDGF-2/sis is capable of acquiring transforming activity when expressed in a cell susceptible to the effects of PDGF. Next, the identification of ras genes, whose normal function remains unknown, is described with special focus on three ras genes of human cells. A discussion on the role of ras genes in naturally occurring human malignancies includes data on identification of a variety of activated ras oncogenes found in numerous tumor types, ras proto-oncogenes of human cells, and ras gene involvement in malignancy. Finally, a review of the possible mechanism of activation of ras oncogenes centers on work wh ich implicates genetic point mutations as the basis for acquisition of malignant properties by ras proto-oncogenes. (74 Refs)

340. GROWTH FACTORS AND CANCER

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Department of Cell Biology. Vanderbilt University School of Medicine. Nashville. Tennessee 37232.
Cancer Res. 46(3):1015-29 1986

Growth factors, defined as polypeptides that stimulate cell proliferation, are major growth-regulatory molecules for cells in culture and probably also for cells in vivo. Nontransformed cells show an absolute requirement for growth factors for proliferation in culture and generally more than one growth factor is required. Under usual culture conditions, growth factors are more rapidly depleted than other media components and thus become rate limiting for proliferation. The loss of or decreased requirement for specific growth factors is a common occurrence in neoplastically transformed cells and may lead to a growth advantage, a cardinal feature of cancer cells. Recent work with transforming growth factors, the platelet-derived growth factor, and oncogenes has produced some insight into the mechanisms through which alterations in growth factor-receptor-response pathways could lead to a growth advantage. Evidence has been derived for autocrine secretion in which the cell produces its own growth factor. Many transformed mesenchymal cells produce PDGF (the product of the c-sis proto-oncogene) and certain transformed cells both produce and respond in a growth-stimulatory manner to TGF beta. With TGF beta, which is a growth inhibitor for certain epithelial and other cell types, the loss of the normal inhibitory response in transformed cells could have the same result as the activation of a growth-stimulatory response. Two proto-oncogenes, erbB and fms, encode growth factor receptors. In the erbB case, the viral erbB aberrant receptor produced is truncated and appears to be constitutively activated without the need for a growth factor. Recent studies suggest that the p21 product of the ras oncogene may be an obligatory intermediate in transducing the growth factor signal. Activation of ras may, therefore, activate the growth factor pathway without the need for either a growth factor or its receptor. The transcription of myc and fos is induced by growth factor stimulation of quiescent cells. The protein products of both are nuclear associated and conceivably could be involved in regulating other genes important in the control of cell proliferation. Activation or inappropriate expression of either myc or fos could produce the same end result as stimulation of a growth factor pathway leading to a growth advantage. Study of the molecular mechanism(s) of growth factor action has just begun. The excitement and attention focused on cellular oncogenes in recent years is now turning toward growth factors, not only as they concern the control of normal cell growth but al so the involvement of growth factor-initiated pathways in the etiology of cancer.(ABSTRACT TRUNCATED AT 400 WORDS) (267 Refs)

341. SYNERGISTIC MECHANISMS IN THE COMBINED CARCINOGENIC ACTION OF RADIATION AND OTHER FACTORS: THE ROLE OF MUTATION AND THE ACTIVATION OF ONCOGENES AND GENES CONTROLLING GROWTH FACTOR SYNTHESIS

Vilenchik MM
Radiobiologia. 24(4):569-71 1984

A hypothesis is proposed on the role in the radiation carcinogenesis of mutation and (or) activation of combination of the several prooncogenes, some of which are homologous on the growth factors or probably to the acain. The antibodies to the products of oncogenes may be used to study mechanisms of carcinogenic action of radiation and others factors or even as diagnostic tool or remedy.

342. PARACRINE ACTION OF TRANSFORMING GROWTH FACTORS

Gol-Winkler R
Clin Endocrinol Metab. 15(1):99-115 1986

Polypeptide growth factors form a class of regulatory molecules which exert their effects by binding to specific receptors present on the cell surface. Most of the time the exact role of these factors in the healthy body is unknown. Some, like PDGF and TGF beta, seem to be involved in wound healing. Others, like EGF, promote epithelial cell growth and differentiation. The site of synthesis of most polypeptide growth factors is unknown. Their target can be identified by detecting the cells which present the specific receptors at their surface. It is though that polypeptide growth factors have a paracrine mode of action. Many different cancerous cells produce polypeptide growth factors and the appropriate receptors. Thus, they are able to stimulate their own growth in an autocrine fashion. Recently, some polypeptide growth factors and receptor genes or cDNAs have been molecularly cloned. Growth factor genes and messengers are much more complex than would be expected from the size of the polypeptide. Some cDNAs have been introduced into bacterial expression vectors and large amounts of the factors have been produced by bacteria. New tools, such as molecular probes and specific antibodies, are thus now available to investigate the production of the growth factors and their receptors. The same tools will facilitate the identification and understanding of the molecular mechanism whereby cancerous cells produce the growth factors and the appropriate receptors simultaneously. The importance of growth factors and receptors in cancer is stressed by the finding that three oncogenes are in fact the genes coding for one growth factor and two receptors. Finally, the molecular probes and the specific antibodies raised against these molecules can be used to identify precisely the growth factor(s) and receptor(s) produced abnormally in cancers. Antibodies that inhibit specifically the interaction of this very growth factor with its receptor could then be developed, thus allowing hum an tumour cell growth to be controlled. (117 Refs)

343. THE MOLECULAR BIOLOGY OF CARCINOGENESIS

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Det Oncol. 2(3):9-18 1984

Fast transforming RNA retroviruses contain genes for viral replication and a gene or genes that transform host cells,

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called the viral oncogene (v-onc). Viral oncogenes are homologous to cellular proto-oncogenes (c-onc). Cellular proto-oncogenes also can initiate cell transformation if they are activated, as evidenced by DNA transfection studies in which DNAs from tumor cells were able to transform normal cells; the transformed cells, when inoculated into immunocompromised mice were able, in turn, to give rise to fibrosarcomas. Transforming DNA sequences have thus been identified in human cancers. Human c-onc can also be activated by gene amplification, by single-point mutation, or by transposition. Slowly transforming retroviruses do not contain a v-onc but stimulate expression of a c-onc. Radiation and chemical carcinogens also may activate a c-onc. Expression of multiple c-onc may be involved, as demonstrated in rat embryo fibroblasts transformed by a human bladder carcinoma c-onc. Two groups of oncogenes have been distinguished, one responsible for establishment (immortalization), the other responsible for tumor growth (transformation). The cellular products of these genes are not known, although those of immortalizing genes are found in the nucleus and those of transforming genes are found in the cell membrane. Some oncogenes are homologous to growth factors or enzymes. In melanoma, specific chromosomal translocations occur, but these are not known to involve oncogenes. Random chromosomal translocations have been reported in a family with the Dysplastic Nevus Syndrome. (39 Refs)

344. THREE STEPS MUTATION MODEL OF CARCINOGENESIS

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University of Calgary, Department of Medical Biochemistry, Alberta, Canada
Med Hypotheses; 14(4):363-71 1984

Based on recent data, this model supposes that benign and malignant tumors arise when a specific mutation takes place in a specific protein-kinase encoded by a proto-oncogene in a pluripotent stem cell or a committed stem cell. Protein-kinases which are regulated by tissue growth stimulating factors, tissue growth inhibiting factors and differentiating factors phosphorylate specific proteins that can derepress specific genes leading to cell division and cell differentiation. Malignant tumors arise when three mutations happen in a same pluripotent stem cell or committed stem cell: positive mutation at the level of tissue growth stimulating factor; negative mutation at the level of tissue growth inhibiting factor; negative mutation at the level of tissue differentiating factor. Higher expression of oncogenes and amplification reflect the relationship between tumor and host. Chromosomal rearrangements are secondary events that can be important because they may involve genes which are normally not involved in cell division and cell differentiation.

345. MECHANISMS INITIATING CELLULAR PROLIFERATION

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Dept. of Pharmacology, Lineberger Cancer Res. Center, The Univ. of North Carolina, Chapel Hill, NC 27514
UT MD Anderson Symp Fundam Cancer Res; 37:31-46 1985

The decreased dependency of transformed cells on serum or serum-derived factors for growth raises the question of how such cells proliferate in the absence of exogenous mitogenic signals. Studies have identified platelet-derived growth factor (PDGF) as the serum component initiating the cell cycle traversal of G0-arrested nontransformed mouse embryonic cells (BALB/c-3T3) and demonstrated the capacity of spontaneously transformed counterpart cells (ST3T3) to proliferate independently of this polypeptide. The role of PDGF and other growth factors in the regulation of cell growth of nontransformed cells is reviewed with emphasis on the mechanism by which PDGF exerts its influence on cells in the prereplicative phase. Cellular transformation and growth factors such as PDGF increase the proliferative capacity of target cells. Evidence is presented showing that some of the proteins encoded by proto-oncogenes, called transforming growth factors, show near-identical structure and activity to known growth factors such as PDGF and may account for the altered growth-factor dependency observed in these cells. PDGF can initiate proliferation by inducing the production of a stable cellular mediator capable of triggering G0/G1 traversal. The search for potential protein mediators of PDGF is reviewed historically and with regard to current efforts to detect proteins selectively synthesized in response to PDGF. Of these proteins, the most extensively reviewed here is pI (MW 29,000), whose growth-related properties are most consistent with its putative role as the cellular/mediator of PDGF. Finally, the possible involvement of protein kinase in bridging the events between the initial interaction of PDGF with target cells and the induction of unique gene products is examined. (68 Refs)

346. CALCIUM. MOLECULAR MECHANISMS OF CELLULAR FUNCTION

Marme D
Abteilung Biophysik, Godecke Forschungsinstitut, Freiburg
Naturwissenschaften; 72(3):113-7 1985

Calcium plays a pivotal role in cellular signal transduction. Precise control and regulation of cytoplasmic free ionic calcium concentration is a prerequisite for intracellular calcium-mediated physiology. Calmodulin is the ubiquitous calcium receptor protein which conveys the information to the calcium-dependent enzymes. Protein kinase C and receptor-stimulated phosphatidylinositol metabolisms are components of another regulatory mechanism where calcium plays an important regulatory role, too. Oncogene products have been found to possess enzymatic activities involved in phosphatidylinositol metabolism. (10 Refs)

347. CYTOSKELETAL EVENTS DURING CALCIUM- OR EGF-INDUCED INITIATION OF DNA SYNTHESIS IN CULTURED CELLS. ROLE OF PROTEIN PHOSPHORYLATION AND CLUES IN THE TRANSFORMATION PROCESS

Marceau N, Suierenga SH
Hotel-Dieu Hospital, Department of Medicine, Laval University, Quebec, Canada.
Cell Muscle Motil; 6:97-140 1985

The possible relationship between cytoskeletal events and growth regulation in response to stimulation by calcium and by growth factors such as EGF can be summarized as follows: An elaborate interaction exists between calcium and serum growth factors, such as EGF, in the initiation of DNA synthesis in quiescent cells. This implies that many processes between the external signals delivered at the cell surface and the sequential intracellular events that lead to chromosomal replication, and ultimately to cell division, must be coordinated in a reproducible manner. It is now apparent that because of its possible role as a dynamic integrator of the cytoplasm, the cytoskeleton could represent the coordinator of the events that lead to replication. Calcium (with its intracellular acceptor, calmodulin) and cAMP (which can act by opposing mechanisms) are extensively involved in the control of the integrity of the cytoskeleton. Distinct protein kinases are activated by calcium/calmodulin, EGF, and cAMP as aspects of the prereplicative response, and many of the substrates for phosphorylation are cytoskeletal proteins. The emerging picture seems to include a direct involvement of these protein kinases in the cascade of regulatory events that

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leads to the initiation of DNA synthesis. Thus, the cytoskeleton has a direct role in the transmission of proliferative signals from external receptor sites to the nucleus. A means by which neoplastic cells can bypass the normal regulatory pathways is proposed in the light of recent data showing that the product of oncogenes are protein kinases or proteins that intimately interact with cellular protein kinases. (168 Refs)

348. ONCOGENES OF SPONTANEOUS AND CHEMICALLY INDUCED TUMORS

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Adv Cancer Res, 36:149-163 1982

The value of experiments involving the process of gene transfer, termed transfection, in resolving questions concerning the role of DNA damage in oncogenic transformation is discussed. Topics reviewed include a model of cellular oncogenes; the retrovirus-associated oncogenes; oncogenes of cells transformed by chemical carcinogens; the transforming genes in 3-methylcholanthrene-transformed cells; types of transformed cells yielding focus-induced DNA; the multiplicity of human oncogenes; and analogies between virus-and non-virus-induced cellular oncogenes. The process of activation of oncogenes, the role of oncogenes in carcinogenesis and maintenance of phenotype, and the proteins encoded by activated oncogenes are discussed. It is unlikely that activation of a cellular oncogene is the only necessary alteration occurring during the conversion of a normal cell into a tumor cell; other genetic and epigenetic controls are probably involved in realization of the oncogenic phenotype. Experiments to date suggest, however, that a multistep process is not involved, and that a single genetic determinant is able to effect the conversion of the recipient cell from normal fibroblast into highly tumorigenic fibrosarcoma cell. It is possible that recipient cells in such experiments already have undergone many necessary predisposing steps, and that only the final alteration is provided by the transfected oncogene. (34 Refs)

349. ANALYSIS OF TRANSFORMING PROTEINS CODED FOR BY VIRAL AND CELLULAR ONCOGENES (MEETING ABSTRACT)

Moelling K, Benter T, Bernling P, Sander T, Heimann B, Pfaff E

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XV Meeting of the European Tumour Virus Group, September 19-24, 1984, Urbino, Italy, p 8, 1984

The gene products of several oncogenes of viral and cellular origin have been analyzed in vitro. It appears that they can be classified in three functional groups: (1) nuclear antigens which bind to DNA in vitro (myc, myb) (2) cytoplasmic proteins which bind to RNA in vitro (mil and raf) (3) protein kinases (src and fps). Data on purified oncogene products of representatives of each of these groups were presented. Oncogene products were purified by immune-affinity column chromatography using specific immunoglobulins. Antibodies were produced against bacterially expressed oncogenes of viral and cellular origin or parts thereof such as v-myc, human-c-myc, v-myb, v-erbB or synthetic peptides. myc and myb-specific oncogene products were purified from avian and human cell lines. The human myc-gene product predominantly resides in the nucleus and binds to DNA in vitro. Whether or not it recognizes specific DNA sequences is under investigation. Studies on purified mil, raf and fps were also mentioned.

350. SUBCELLULAR LOCATIONS OF RETROVIRAL TRANSFORMING PROTEINS DEFINE MULTIPLE MECHANISMS OF TRANSFORMATION.

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Adv Viral Oncol, 4:269-306 1984

Oncogenic retroviruses, their onc-encoded polypeptides, and the intra-cellular distribution of these transforming proteins are described. The onc gene proteins are presented in groups derived from avian sarcoma viruses, mammalian tumor viruses, and avian acute leukemia viruses. Because the cellular location and biochemical properties of the src gene product of Rous sarcoma virus (RSV) have been more extensively studied, it is discussed more thoroughly. Several subcellular compartments that host distinct onc gene products are identified and the transformation mechanism is discussed. These locations include the cytoplasm, plasma membrane, adhesion plaques and the cytoskeleton. Several proteins, thought to be involved in transformation, and their gene sites are discussed, including 'yes' gene proteins of the Esh sarcoma virus, the fps gene proteins of the FuSV, PRC (Poultry Research Center) II, IIp and IV and the UR1 viruses, and the ros gene proteins of the transforming virus UR-2. Genes thought to code for transforming proteins are discussed in a variety of systems including mammalian tumor viruses and avian acute leukemia viruses. Finally, a summary of transforming proteins along with their respective onc genes and virus designation is presented with concluding remarks about v-onc vs c-onc genes. (152 Refs)

351. VIRAL MECHANISMS OF CARCINOGENESIS

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IARC Sci Publ: (32):307-16 1982

Several kinds of virus can cause cancer. The various viruses with oncogenic potential probably act through different mechanisms and depend on different host factors. The tumour viruses with small genomes are best understood and may be regarded as transposable genetic elements. By integrating into host cell chromosomal DNA, they may cause mutations and chromosomal rearrangements that predispose to cancer. The insertion of viral promoter sequences adjacent to certain host genes can lead to ectopic gene expression resulting in neoplasia. There is growing evidence, however, that the most strongly oncogenic strains of small DNA and RNA viruses carry 'oncogenes' that encode 'transforming' proteins. Papovavirus early proteins interact with cellular proteins involved in the control of cell proliferation. Many, but not all, of the transforming proteins of retroviruses are located at the plasma membrane and exhibit protein kinase activity, phosphorylating tyrosine residues. Most of the oncogenes of retroviruses studied so far are derived from cellular genes which may play important roles in regulating normal cell proliferation and differentiation. Retrovirus oncogenes may therefore be regarded as kidnapped host factors placed under the control of infectiously transmitted promoter elements. A variety of other host factors and exogenous cofactors affect the course of viral carcinogenesis, from influencing initial infection to immunological responses to chronic infection. Lastly, the endogenous viral genomes, which are inherited as host genetic elements, should themselves be considered as host factors that interact with exogenous carcinogens such as chemicals and ionizing radiation.

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352. POSSIBLE MECHANISMS OF THE TRANSFORMING AND TUMORIGENIC EFFECTS OF DNA-CONTAINING ANIMAL VIRUSES

Gartel' AL. Tikhonenko TI
Mol Biol (Mosk): 19(3):581-96 1985

The literature devoted to oncogenic action of DNA-containing animal viruses and their role in the development of human neoplasias are reviewed. The regularities of persistence and expression of genetic material of DNA-containing viruses in transformed and tumor cells are comprehensively analyzed. The mechanisms of recombination of cellular and viral DNA during cell transformation as well as the specificity of integration of viral DNA into the host genome are considered. The functions and mechanisms of transforming and tumorigenic action of the products of oncogens of DNA-containing viruses of different groups are discussed. The data on the cell transformation by some DNA-containing viruses without oncogene expression are represented. The mechanism of cell transformation by DNA-containing viruses related to the activation of cellular oncogens is discussed. (132 Refs)

353. ONCOGENES OF AVIAN LEUKEMIA VIRUSES

Hayman MJ
Imperial Cancer Research Fund Laboratories. St. Bartholomew's Hosp., London EC1A 7BE, England
Life Sci Res Rep: 30:163-78 1985

Characteristics of the oncogenes of avian acute leukemia viruses (ALVs) are reviewed. The ALVs are divided into three classes, based on their oncogenes, as follows: the erb oncogene of AEV-ES4 and AEV-H viruses (which cause erythroblastosis and sarcoma); (2) the myc oncogene of MC29 virus (which causes myelocytoma, carcinoma and endothelioma), of CM11 virus (which causes myelocytoma), and of OK10 and MH2 viruses (which cause endotheliomas); and (3) the myb oncogene of AMV (which causes myeloblastosis) and of E26 virus (which causes myeloblastosis and erythroblastosis). There is no single mechanism by which these oncogenes function, but there may be only a limited number of strategies that can be employed to induce malignant transformation. Oncogenes exert their action in various parts of the cell, with their products being found in the plasma membrane, under the plasma membrane, in the cytoplasm, and within the nucleus. Oncogenes may represent all kinds of molecules, such as growth factors, growth factor receptors, intracellular regulatory molecules such as protein kinase, and nuclear proteins that directly influence transcription and division. Three mechanisms that could account for the uncontrolled action of these oncogene products in the transformed state include the following: there may be a transforming domain of the protein in the viral version of the oncogene, but without a regulatory domain that normally accompanies it; it may be that the oncogenic protein is not mutated at all, but that the problem is inappropriate expression; a third possibility is over-expression of the gene product, a hypothesis that is less popular now than formerly, but that must still be considered viable. The three mechanisms may not be mutually exclusive, and it is suggested that an example may be found for the applicability of all three mechanisms. (44 Refs)

354. [GROWTH FACTOR RECEPTOR GENES AND ONCOGENES] (44 Refs)

Shimizu N
Nippon Rinsho: 44(1):194-201 1986

Keywords (MeSH): Animal; Antigens, Neoplasm/analysis; Growth Substances/*metabolism; *Oncogenes; Platelet-Derived Growth Factor/metabolism; Receptors, Endogenous Substances/*genetics; Receptors, Insulin/genetics; Review; Transferrin/metabolism.

355. GROWTH FACTORS IN CANCER AND THEIR RELATIONSHIP TO ONCOGENES (209 Refs)

Salomon DS, Perroteau I
Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, Maryland 20205.
Cancer Invest: 4(1):43-60 1986

Keywords (MeSH): Bombesin/genetics; Cell Line; Cell Transformation, Neoplastic; Epidermal Growth Factor-Urogastrone/genetics; Gastrins/genetics; Growth Substances/*genetics; Human; Neoplasms/*familial and genetic; Nerve Growth Factors/genetics; *Oncogenes; Platelet-Derived Growth Factor/genetics; Receptors, Endogenous Substances/genetics; Review; Somatomedins/genetics; Transferrin/genetics.

356. PROTO-ONC GENE PRODUCTS AS DIFFERENTIATION ANTIGENS (A REVIEW) (128 Refs)

Foldes I, Minarovits J
Microbiological Research Group of the National Institute of Hygiene, Budapest, Hungary.
Acta Microbiol Hung: 31(4):325-34 1984

Keywords (MeSH): Animal; Antigens/*genetics; Base Sequence; *Cell Differentiation; Cell Transformation, Viral; Gene Expression Regulation; Genes, Viral; Human; Nucleic Acid Hybridization; *Oncogenes; Retroviridae/genetics; Review; Transcription, Genetic; Translation, Genetic; Viral Proteins/genetics.

357. PROTOONCOGENE EXPRESSION DURING THE CELL CYCLE (142 Refs)

Kaczmarek L
Temple University Health Science Center Department of Pathology, Philadelphia, Pennsylvania.
Lab Invest: 54(4):365-76 1986

Keywords (MeSH): Actins/genetics; Animal; Calmodulin/genetics; *Cell Cycle; Cell Differentiation; Cell Division; DNA/biosynthesis; Gene Expression Regulation; Glycolysis; Glycoproteins/genetics; Heat-Shock Proteins/genetics; Histones/genetics; Human; Interferon Type II/genetics; Mitogenic Factors, Lymphocyte/genetics; Ornithine Decarboxylase/genetics; Peptides/genetics; Proto-Oncogene Proteins, Cellular/*biosynthesis/genetics; *Proto-Oncogenes; Receptors, Endogenous Substances/genetics; Receptors, Immunologic/genetics; Review; Tetrahydrofolate Dehydrogenase/genetics; Thymidine Kinase/genetics; Thymidylate Synthetase/genetics; Tubulin/genetics.

358. THE VIRAL TYROSINE PROTEIN KINASES

Sefton BM
Curr Top Microbiol Immunol: 123:39-72 1986

Keywords (MeSH): Abelson Leukemia Virus; Animal; Avian Sarcoma Viruses; Cell Compartmentation; *Cell Transformation, Viral; Myristic Acids/metabolism; Oncogene Proteins, Viral/*metabolism; Phosphoinositides/physiology; Phosphorylation; Protein-Tyrosine Kinase/*metabolism; Receptors, Endogenous Substances/genetics; Retroviridae Proteins/metabolism; Retroviridae/*enzymology; Substrate Specificity; Tyrosine/analogs and derivatives/metabolism.

359. HOMOLOGY AMONG ONCOGENES

Van Beveren C, Verma IM
Curr Top Microbiol Immunol: 123:73-98 1986

Keywords (MeSH): Amino Acid Sequence; Animal; Cell Nucleus/physiology; Comparative Study; Genes, Structural; Growth Substances/physiology; Guanine Nucleotide Regulatory Protein/genetics; Oncogene Proteins, Viral/*genetics; *Oncogenes; Protein-Tyrosine Kinase/*genetics; Proto-On-

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cogene Proteins, Cellular/*genetics; Receptors, Endogenous Substances/genetics; Sequence Homology, Nucleic Acid.

360. THE PROTEINS OF ONCOGENES
Hunter T
Sci Am: 251(2):70-9 1984

Keywords (MeSH): Animal; Cell Transformation, Neoplastic/*metabolism; Cell Transformation, Viral; DNA/metabolism; Epidermal Growth Factor-Urogastrone/physiology; Guanosine Triphosphate/metabolism; Human; Membrane Proteins/physiology; Neoplasm Proteins/*physiology; Nucleoproteins/physiology; *Oncogenes; Platelet-Derived Growth Factor/physiology; Protein Binding; Protein Kinases/metabolism; Receptors, Endogenous Substances/metabolism; Retroviridae/genetics; Translation, Genetic; Tyrosine/analogs and derivatives/metabolism; Viral Proteins/physiology.

361. ONCOGENES AND PROTO-ONCOGENES: HOW DO THEY DIFFER? (160 Refs)
Hunter T
Molecular Biology and Virology Laboratory, Salk Institute, San Diego, Calif.
JNCI: 73(4):773-86 1984

Keywords (MeSH): Animal; Cell Cycle; *Cell Transformation, Neoplastic; Comparative Study; Genes, Viral; Growth Substances/genetics; Guanine Nucleotide Regulatory Protein/genetics; Human; Nucleoproteins/genetics; *Oncogenes; Protein Kinases/genetics; Retroviridae/genetics; Review; Transcription, Genetic.

362. EXPRESSION OF CELLULAR ONCOGENES (250 Refs)
Muller R, Verma IM
Curr Top Microbiol Immunol. 112:73-115 1984

Keywords (MeSH): Animal; Cats; Cell Transformation, Neoplastic; Comparative Study; Gene Expression Regulation; *Genes, Viral; Human; Mice; *Oncogenes; Oncogenic Viruses/*genetics; Review; Tumor Virus Infections/etiology.

363. HUMAN PROTO-ONCOGENES, GROWTH FACTORS, AND CANCER (74 Refs)
Aaronson SA, Robbins KC, Tronick SR
National Cancer Institute, Bethesda, Maryland.
Symp Fundam Cancer Res: 37:241-55 1984

Keywords (MeSH): Animal; Base Sequence; *Cell Transformation, Neoplastic; Cell Transformation, Viral; Gene Expression Regulation; Growth Substances/*genetics; Human; *Oncogenes; Platelet-Derived Growth Factor/genetics; Review.

364. GROWTH FACTORS: MECHANISM OF ACTION AND RELATION TO ONCOGENES
Heldin CH, Westermark B
Department of Medical and Physiological Chemistry, University of Uppsala, Sweden.
Cell: 37(1):9-20 1984

Keywords (MeSH): Animal; *Cell Transformation, Neoplastic; Epidermal Growth Factor-Urogastrone/physiology; Growth Substances/*physiology; Human; Insulin/physiology; Mitosis; Neoplasms/etiology; *Oncogenes; Platelet-Derived Growth Factor/physiology; Protein Kinases/metabolism.

365. GROWTH FACTORS, ONCOGENES AND TRANSFORMATION. PART I: GROWTH FACTORS AND CELL CYCLE CONTROL (189 Refs)
Paul D
Department of Toxicology, Hamburg University Medical School, Federal Republic of Germany.
Arzneimittelforschung: 35(4):772-9 1985

Keywords (MeSH): Animal; Cell Division; *Cell Transformation, Neoplastic; Cell Transformation, Viral; Cells, Cultured; Epidermal Growth Factor-Urogastrone/physiology; Gene Expression Regulation; Growth Substances/*physiology; Hormones/biosynthesis; Human; Lymphocyte Transformation; Mitogens/pharmacodynamics; *Oncogenes; Oncogenic Viruses/genetics; Receptors, Endogenous Substances/physiology; Review.

366. ROLES OF GROWTH FACTOR ACTIVITIES IN ONCOGENESIS
Deuel TF, Huang JS
Jewish Hospital at Washington University Medical Center, St Louis, MO.
Blood: 64(5):951-8 1984

Keywords (MeSH): Amino Acid Sequence; Blood Platelets/metabolism; Cell Transformation, Neoplastic; Growth Substances/*physiology; Human; *Oncogenes/classification; Platelet-Derived Growth Factor/physiology; Protein Kinases/metabolism; Simian Sarcoma Viruses/growth and development; Structure-Activity Relationship; Temperature; Transcription, Genetic; Viral Proteins/genetics/physiology.

367. LEUKEMIA, GROWTH FACTORS AND ONCOGENES
Forchhammer J
Leuk Res: 8(2):161-3 1984

Keywords (MeSH): Animal; Gene Expression Regulation; Growth Substances/*physiology; Human; Leukemia/*physiopathology; *Oncogenes; Retroviridae/physiology.

368. CONFORMATIONAL PROPERTIES OF ONCOGENE PRODUCTS
Kieber-Emmons T, McDonald J, Rein R
Unit of Theoretical Biology, Roswell Park Memorial Institute, Buffalo, New York 14263.
Prog Clin Biol Res: 172A:453-64 1985

Keywords (MeSH): Amino Acid Sequence; Neoplasm Proteins; *Oncogenes; *Protein Conformation.

369. SEQUENCE HOMOLOGIES OF ONCOGENE PROTEINS: A CRITICAL REVIEW
Kanehisa M, DeLisi C
Laboratory of Mathematical Biology, National Cancer Institute, Bethesda, Maryland 20205.
Prog Clin Biol Res: 172A:443-52 1985

Keywords (MeSH): Amino Acid Sequence; Cell Transformation, Neoplastic; Neoplasm Proteins/analysis; *Oncogenes; Proteins/*analysis.

370. ROLE OF TYROSINE PHOSPHORYLATION IN MALIGNANT TRANSFORMATION BY VIRUSES AND IN CELLULAR GROWTH CONTROL
Hunter T, Cooper JA
Prog Nucleic Acid Res Mol Biol: 29:251-82 1983

Keywords (MeSH): Animal; *Cell Transformation, Neoplastic; Genes, Viral; Oncogenes; Phosphoproteins/isolation and purification; Phosphorylation; Protein Kinases/*metabolism; Retroviridae/*genetics.

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371. TYROSINE PROTEIN KINASES AND THEIR SUBSTRATES: AN OVERVIEW (94 Refs)

Hunter T, Cooper JA

Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California

Adv Cyclic Nucleotide Protein Phosphorylation Res, 17:443-58, 1984

Keywords (MeSH): Lactate Dehydrogenase/metabolism; Muscle Proteins/metabolism; Oncogenes; Phosphoglycerate Mutase/metabolism; Phosphopyruvate Hydratase/metabolism; Phosphorylation; Protein Kinases/genetics/*physiology; Receptors, Endogenous Substances/physiology; Retroviridae/enzymology; Review; Tyrosine/analogs and derivatives/analysis; Viral Proteins/physiology.

372. PROTEIN-TYROSINE KINASES (239 Refs)

Hunter T, Cooper JA

Molecular Biology and Virology Laboratory, Salk Institute, San Diego, California 92138

Annu Rev Biochem, 54:897-930, 1985

Keywords (MeSH): Amino Acid Sequence; Animal; Binding Sites; Epidermal Growth Factor-Urogastrone/*physiology; Human; Lipids/metabolism; Oncogenes; Phosphorylation; Protein Kinases/antagonists and inhibitors/classification/*metabolism; Protein Processing, Post-Translational; Receptors, Endogenous Substances/*physiology; Retroviridae/*enzymology; Review; Serine/metabolism; Substrate Specificity; Threonine/metabolism; Tyrosine/*metabolism; Viral Proteins/metabolism.

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SUBJECT INDEX

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